Project no 010284-2.

EDEN

Emerging diseases in a changing European environment

Integrated Project

Sub- Priority 6.3: Sustainable development, Global Change and Ecosystems

Periodic activity report, fourth 12-month period

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Project coordinator organisation name : CIRAD

Submitted [draft 2]

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List of Abbreviations

The table doesn't include partner short names

AFR	Africa platform
AG	EDEN Advisory Group
ASTER	Advanced Space borne Thermal Emission and Reflection Radiometer
AVHRR	Advanced Very High Resolution Radiometer
CDC	(US) Centre for Diseases Control
CDS/CSR	WHO, Department of Communicable Diseases Surveillance and Response
DMEWS	Disease Monitoring and Early Warning Systems
D_x	Project deliverable x
EC	European Commission
ECDCP	European Centre for Disease Control and Prevention
ECSITE	European Collaborative for Science, Industry and Technology Exhibitions
EDEN	Emerging Diseases in a Changing European Environment
EO	Earth observation
EPBRS	European Platform for Biodiversity Research Strategy
EPIDEMIO	Earth Observation in Epidemiology
ESA	European Space Agency
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FP6	6th framework program
GIS	Geographical Information System
GM	Ground measured (as opposed to remotely sensed)
GMES	Global Monitoring for Environment and Security
GMFS	Global monitoring for food security
GPS	Global Positioning System
H&E	Health and Environment Research
HIT	Horizontal Integration Team
HR	High Resolution (Satellite imagery)
IP	Integrated Project
IDEAS	Integrated Data Exchange and Archiving System
LEI	Leishmaniasis
LR	Low Resolution (Satellite imagery)
MAL	Malaria
MERIS	Medium Resolution Imaging Spectrometer Instrument

METEOSAT	European Meteorological Satellite
MODIS	Moderate Resolution Imaging Spectroradiometer
M_{xx}	Month x
NASA	National Aeronautics and Space Administration
NDVI	Normalized Difference Vegetation Index
NOAA	National Oceanic and Atmospheric Administration
OIE	World Organisation for Animal Health (formerly: Office International des Epizooties)
PC	EDEN Project coordinator
PENCIL	European School net
PhD	Doctor of Philosophy
PS	EDEN Project secretariat
REX	Network of excellence
R&D	Research and Development
Robo	Rodent borne diseases
RS	Remote sensing
RTD	Research and Technical Development
SARS	Severe Acute Respiratory Syndrome
SC	EDEN Steering Committee
SD	EDEN Strategy Document
SP	Sub-Project
SYNTHESYS	The Worlds Largest Network of Natural History Institutions
T&P	Tools and Policies Development
TBD	Tick borne diseases
TBE	Tick borne encephalitis
TM	Thematic Mapper
UK	United Kingdom
USA or US	United States of America
WHO	World Health Organisation of the United Nations
WNV	West Nile virus
WP	Work package
y_x	Project year 1-5

Executive Summary

In recent years, several vector-borne, parasitic or zoonotic diseases have (re)-emerged and spread within Europe with major health, ecological, socio-economical and political consequences. Most of these outbreaks are linked to global and local changes caused by either climate change, human-induced landscape changes or the direct impact of human activities. The EDEN IP (Emerging Diseases in a changing European eNvironment) offers a unique opportunity to prepare for uncertainties about the future of the European environment by exploring the potential impact of environmental and other changes on human health.

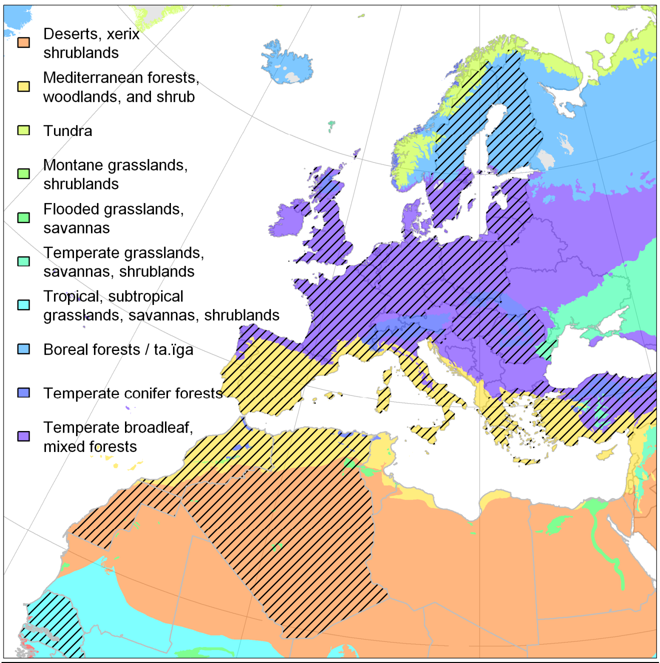

Fig. Coord-01. Biomes covered by the EDEN project. Shaded countries are involved in EDEN. Data source for biomes: Olson et al. (2001). Terrestrial ecoregions of the world: A new map of life on Earth.* BioScience*, 51: 933-938.

The goals of EDEN are to identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change, which can influence the spatial and temporal distribution and dynamics of human pathogenic agents. Through a coordinated approach, the project is developing and co-coordinating, at the European level, a set of generic methods, tools and skills such as predictive disease emergence and spread models, and identifies requirements for early warning, surveillance and monitoring tools and scenarios, to be used by decision makers for risk assessment, decision support for intervention and public health policies at either the EU, the national or regional level. Part of EDEN's innovation is to combine spatial data (earth observation data, GIS etc) with epidemiological data.

Recognition that the environment may affect the risk of both infectious and vector borne diseases is not new, but recently, a better understanding has been reached of the mechanisms that underlie the complex interactions between infectious agents, the hosts and the biological and physical environment. Vector-borne zoonoses tend to be the most ecologically complex infectious diseases in which environmental change may have the greatest number and diversity of effects, some promoting transmission and others diminishing it. Habitat and species losses may reduce the normal buffering within ecosystems leading to disease outbreaks. Finally, the juxtaposition of new vectors, hosts and parasites within disturbed ecosystems provides a potential for the evolution of novel transmission pathways and thus new 'emerging diseases'.

The EDEN project integrates research in 49 leading institutes from 24 countries with the combined experience and skills to reach the project's common goals. The eco-geographical diversity of the project area covers all relevant European eco-systems (fig. Coord-01) from the Arctic Circle in the North to the Mediterranean Basin and its link with West Africa in the South, and the Atlantic Ocean in the West to the Danube Delta in the East.

EDEN has selected a range of indicator human diseases that are especially sensitive to environmental changes and are studied within a common scientific framework (involving landscapes, vector and parasite bionomics, public health, and animal reservoirs). Some of these diseases are already present in Europe (tick- and rodent-borne diseases, leishmaniasis, West Nile fever), some were present historically (malaria) and so may re-emerge, whilst others are found on the fringes of Europe, like Rift Valley fever which is present in Africa and the Middle East. The diseases are studied in so-called "vertical" sub-projects (SP). Each of these subprojects conducts health-environment epidemiological studies, i.e. studying patterns and processes of diseases as part of their environment, organized in the same set of five work-packages: WP1 – Landscapes, biotopes and habitats, WP2 – Vector bionomics and competence, WP3 – Public health and human activities, WP4 – Animal reservoirs, and WP5 – Data management, analysis and modelling. The integration of these epidemiological studies is achieved through a series of shared horizontal activities (horizontal integration teams, HIT) including: (i) Data-management and information systems, (ii) Remote sensing tools, both High resolution environmental change and Low Resolution spatial modeling (iii) Disease transmission modeling, and (iv) Biodiversity monitoring and assessment.

During the 4th reporting period work focussed on completing field activities as well as strengthening data analysis and modelling. Several problems were overcome, such as the recruitment of short-term positions and postdocs: (i) a complementary SP Leishmaniasis position, to prepare data for modelling, (ii) the delayed recruitment of the HIT LRRS postdoc, to model the impact of environmental changes on disease emergence, and (iii) a complementary HIT Mathematical modelling postdoc, to complete the work started with the PhD thesis of Nienke Hartemink. The two complementary posts were made possible by internal reassignment of EDEN funds. Whilst all EDEN databases are not yet completed and available as planned (mainly EDEN AFR and LEI) contingency plans have been activated to alleviate this.

The overall scientific achievement of the project is excellent (fig. Coord-02) with ca. 130 publications in the peer-review system as per December 2008 (75 in December 2007).

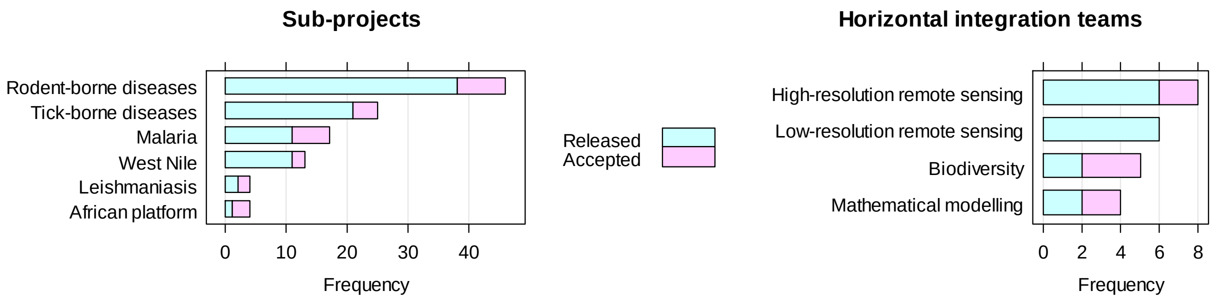

Fig. Coord-02. Number of publications released with an EDEN number as from the beginning of the project

The "Malaria" and "Tick-borne diseases" sub-project have completed very comprehensive studies on several of their field sites, and elaborated different types of predictive risk models. The "Rodent-borne diseases" sub-project has provided the scientific community with an impressive set of high-level publications in all their activity domains, and is now engaged in very promising modelling work. A critical phase has been overcome by the "Leishmaniasis" sub-project. Though few publications were released up to now, an ambitious collaboration has begun with all the horizontal integration teams, to also produce leishmaniasis and vectors distribution and risk maps.

The last year of the project will focus on encouraging partners to publish all their results, and producing the expected integrative papers to reach the initial goals of EDEN: elaboration of generic models for the emergence of vector-borne diseases in Europe, and categorisation of European ecosystems according to the risk of vector-borne disease emergence. The final event organised by EDEN will be an international conference on the impact of environmental changes on emerging, vector-borne diseases. It will be held in Montpellier (France), in May 2010. The proceedings of this conference will be the final version of the EDEN strategy document which summarizes the changes achieved in the state of the art.

A lot of efforts were devoted to EDEN dissemination during 2008. EDEN scientists and members of the steering committee were heavily mobilized to attend a variety of international meetings and workshops, in particular with public-health agencies such as WHO and ECDC, and in world conferences such as the United Nations Climate Change Conference in Poznan, Poland. Several master courses were developed and given to students of European and international origins (with a special attention for Africa). International workshops were also organised where a large floor was given to EDEN scientists, for presenting their methods, tools and results. Skills of EDEN partners were utilized to conduct an expert consultation on behalf of ECDC (European Centre for Disease Prevention and Control, Stockholm): "Assessment of magnitude and importance of vector-borne diseases in Europe". Discussions were pursued with ESA and DG-Sanco to develop applications combining spatial technologies and EDEN methods and results to disease monitoring and early warning systems. FP7 projects began, involving EDEN scientists: e.g., ArboZooNet, a coordination action for the diagnosis and surveillance of several vector-borne diseases. We are also actively involved in the preparation of replies to released FP7 calls, and were consulted as "think tank" to prepare future calls.

In conclusion, studies on specific disease patterns and processes (health-environment research) conducted during the first half of the project have already enabled the development of integrative disease transmission models. In some cases (e.g., malaria in Camargue, France), these models may be readily translated into tools to assist decision support and to feed dissemination. We have good hope that this type of result will be available for wider areas and other diseases before the end of EDEN. Therefore, reaching the ultimate objectives of EDEN is realistic within the (extended) time-frame: (i) develop generic disease models – including risk maps, and (ii) propose tools and scenarios for disease monitoring and early warning systems.

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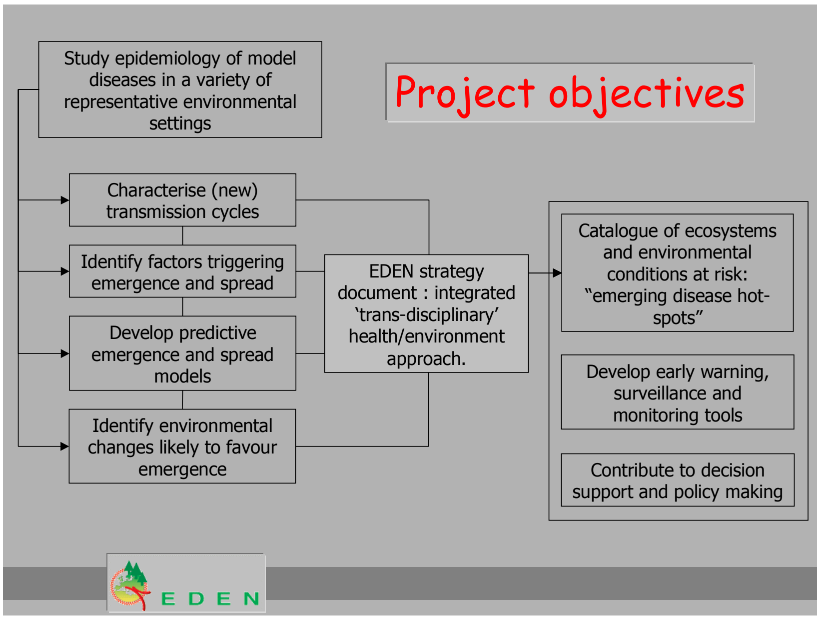
Section 1 – Project objectives and major achievements

1.1 Overview of the general objectives of EDEN

In order to assess global change-driven factors linked to the risk of introducing or spreading emerging human diseases in Europe, the goal of EDEN is to identify, evaluate and catalogue European ecosystems and environmental conditions linked to the effect global change in Europe, which can influence the spatial and temporal distribution and dynamics of pathogenic agents. A coordinated European approach was adopted to provide predictive emergence and spread models including global and regional preventive, early warning, surveillance, and monitoring tools and scenarios. Such tools will have a major impact on improved EU policy development and decision making.



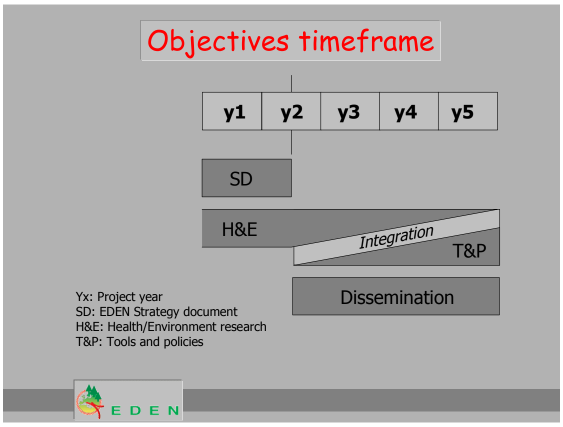


Fig. 1.1 – EDEN Objectives and overall timeframe

The general objectives of the EDEN project are related both to scientific innovations and knowledge improvement on the epidemiological processes involved in the emergence and spread of diseases in a changing environment, and to the methodological development of tools for risk assessment, early warning and policy making. In chronological order this translates as follows:

1. Health-environment research objectives. To describe the epidemiological cycles of selected candidate diseases (see Table 1 below) in a variety of representative environmental settings through an integrated and multidisciplinary approach.
    a. To characterize the infectious agents most likely to emerge in Europe, and the competence and capacity of potential vectors, hosts and reservoirs likely to integrate, perpetuate or spread new functioning disease cycles.
    b. To identify intrinsic and extrinsic factors triggering or modulating emergence and spread in Europe and the endemic disease areas: *i.e* change indicators and risk factors, further referred to as 'indicators'.
    c. To develop and implement methodologies for Pan-European predictive emergence and spread models.
    d. To examine current and (expected) future changes in the European environment likely to favor the emergence or re-emergence of vector-borne diseases.

   EDEN strategy for integration. To develop and apply an EDEN strategy proposing an innovative integrated 'trans-disciplinary' health/environment approach for the unified analysis and exploitation of the various EDEN health-environment research outputs. This strategy aims at the development of generic tools (see Fig 1.1) based on the description and follow up of the set of change indicators and risk factors extracted from the study of disease patterns and processes. A major expected output is to define new methodologies combining statistical approaches and biological models in the definition of these indicators. A particular effort is put on the involvement of environmental sciences.

2. Tools and policies. To develop as stated in the Strategy description and make available to the EDEN and international community a set of generic tools for risk assessment and decision making (maps, risk indicators, scenarios) enabling improved public health decision making at the EU and country level, and more specifically:
    a. To catalogue ecosystems and environmental conditions considered, or predicted, to be at risk ("emerging disease hot-spots").
    b. To develop preventive, early warning, surveillance and mitigation tools and to examine future 'what if' scenarios at different spatial and temporal scales (local to global).
    c. To contribute to decision support and policy making through collaborative initiatives with relevant groups.
3. Dissemination. To promote, through a coordinated European approach, the dissemination of information through awareness-raising and communication in line with social demand from the general public, user groups and the scientific community through: website, leaflet, newsletter, workshops and international meetings, articles and papers, collaborative initiatives, etc.

To achieve these objectives the EDEN Scientific Committee has selected a series of 'indicator diseases' (i) with a strong link with the environment, (ii) currently (at risk) of (re-) emerging or spreading due to environmental and other changes, (iii) representative as a group of a wide geographical range of (changing) eco-systems (iv) representative of the main epidemiological processes involved in emergence.

Table 1 – Selected pathogen groups

Tick-borne pathogens – causing diseases already present in Europe that have shown significant recent increases in incidence, at least partly due to changes in human behaviour in relation to the environment.
Rodent-borne viruses (hanta, arena, cowpox) – widespread but under-reported diseases within Europe, with strong links with habitat and landscape structures.
Leishmaniasis – persistent on the southern fringes of Europe and beyond (southern Mediterranean basin), with the potential to expand as environments change.
West Nile Virus – periodic and occasionally severe local outbreaks, especially on the eastern fringes of Europe which currently are showing strong associations with landscape patterns but also (the USA experience) potential for explosive spread.
Malaria – an ancient scourge of Europe, currently now on her southern and eastern fringes, with the potential for re-emergence following environmental changes.
African source diseases –new strains of West Nile Virus and new diseases such as Rift Valley Fever may be introduced to Europe from tropical regions linked by bird and other (e.g. traded livestock) migratory routes to Europe.

Integration of pathogen studies through the development and application of generic tools and is achieved through a series of horizontal activities managed by 'Horizontal Integration Teams (HIT)'. These include: (i) Data-management and information systems, (ii) Remote sensing tools, both High resolution environmental change and Low Resolution spatial modeling (iii) Disease transmission modeling, and (iv) Biodiversity monitoring and assessment.

The selected diseases are used as applications and horizontal activities bring forward the development of new methodologies for an integrated health-environment approach and innovative indicator driven policy tools. An EDEN strategy document v1.0 was elaborated during year 1 by the EDEN Steering Committee (SP and HIT leaders) with back-up from the Advisory Group (International experts and specialists).

On the long term the EDEN objectives will emphasize improved Public Health policy making. For the selected diseases EDEN will (i) contribute to the area-wide understanding of past and present epidemiological events, (ii) enable the development of spatial and temporal prediction models of amplification and spreading risk, and (iii) contribute to the establishment of improved public health policies at the sub-national, national and regional level. A major expected output is the extension of the EDEN approach to other "similar" diseases. The idea being that developed integrated approaches documented in the EDEN strategy document and disseminated through scientific publications (and other means as per dissemination strategy) may serve as an example for studying diseases transmitted by the same vectors as studied by EDEN or other mainly but not exclusively vector or rodent borne diseases. Examples include: Congo Crimean Fever, Chikungunya, bluetongue (and other animal diseases transmitted by *Culicoides sp.*), plague, dengue, avian influenza…

Through the integration and dissemination of this information EDEN will trigger the development of more generally applicable scenarios enabling (i) the delimitation of quantified disease risk areas, i.e. ecosystems at risk, (ii) the monitoring of temporal risk windows linked to eco-climatic events, (iii) the quantification of exposure to key epidemiological factors, and (iv) the identification of population groups at risk (behavioral, professional).

Furthermore EDEN outputs will significantly contribute to the development of new decision support tools which will enable the implementation of adapted monitoring and control strategies at the local level, the more efficient allocation of efforts and resources at a larger scale and the improved analysis of the impact of land-use planning and reallocation strategies resulting from changes in the agriculture policies and urban/peri-urban demography.

Finally through its dissemination network EDEN will improve public awareness of emerging disease risks and the rational management.

Given the complexity of the EDEN project it is important at this stage to recall the work-page structure of the project (Fig 1.2).

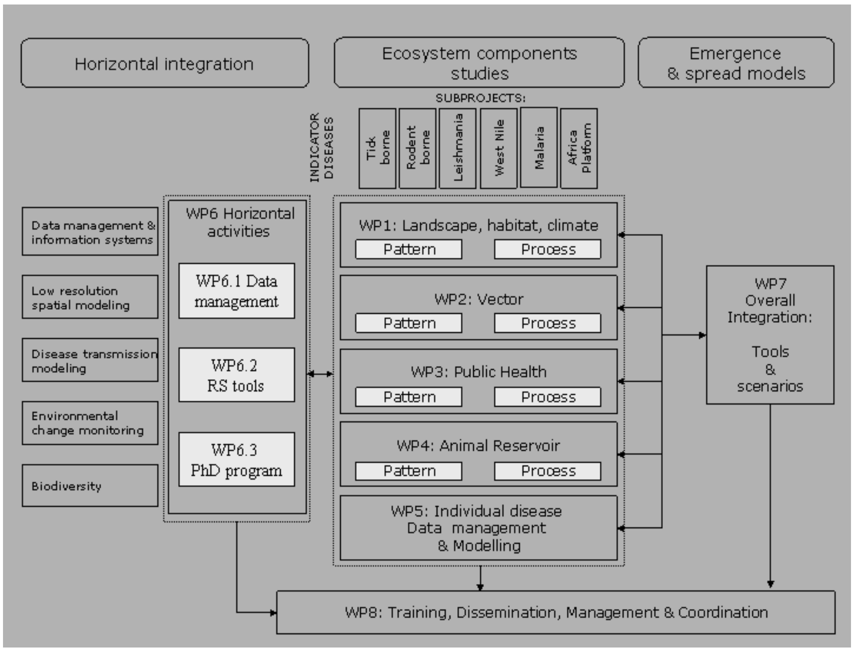


Fig 1.2 **- EDEN workpackage structure**

1.2 EDEN's relation to the state of the art

The state of the art in most EDEN disciplines is one in which we have some idea of patterns and are now working on processes. Thus land-use and land-cover monitoring has moved from the identification of the patterns of land-use change[1,2] to a study of the underlying processes involved[3]. Predictions of the effects of global warming on vector-borne diseases are based on a matching of current patterns to climatic variables now and into the future[4]. We recognize that we need to work on processes but these are still poorly understood for most/all vector-borne diseases[5-6]. Careful analyses of historical and more recent changes in both malaria and tick-borne diseases show that a variety of factors – agricultural, sociological, economical and climatic – contributed to changes in disease patterns over time, with no single one of over-riding importance[7-10]. What each of these examples illustrates is that a cohesive, integrated approach is more likely to give us the right answer than ideas emerging from separate scientific disciplines about the reasons for changes in human disease patterns. Such a cohesive integrated approach must be within a quantitative modeling framework, and EDEN includes world-recognized teams working on all aspects of modeling host-parasite systems; ecosystems[11], vertebrate hosts[12], vectors[13], pathogens and pathogen dynamics through space and time[14].

We envisage a future in which the generic tools developed by EDEN will form the basis of a rapid scientific response methodology (involving both experts and methods) for new and emerging diseases within Europe – essentially an intelligent monitoring and surveillance arm for the European Center for Disease Prevention and Control (ECDC). Currently no Disease Monitoring and Early Warning Systems (DMEWS) exist, in any shape or form, except in the vaguest of outlines and for relatively few diseases. EDEN's bold ambition is to contribute to a multi-DMEWS system on a solid scientific foundation running from the theoretical understanding of the reasons for the emergence of new diseases in vulnerable ecosystems, through an understanding of complex disease dynamics, to the practical application of such knowledge to disease control. We do not know the precise shape of this future yet: EDEN presents a chance to Europe to invest in some of the 'brightest and best' researchers to ensure success with this vision, and in doing so to advance significantly the state-of-the-art and put Europe firmly at the forefront of DMEWS.

1.3 Summary of the main recommendations from previous review

"The project is rated good to excellent.

- …the most serious problem was the 9-month delay in payment of the last interim instalment of the grant by the Commission… To ensure that all sub-projects have adequate time to complete all their objectives the **Reviewers recommend the Commission responds favourably to any request from the EDEN management for a *no-cost* extension to the contract of up to 1 year.** This would provide for one further season for field work to be undertaken where additional data would benefit the modelling exercises and help improve accuracy of the distribution and risk maps.
  Following reviewers' recommendations, a 7-month no-cost extension was requested to the Commission.

- …it is suggested that EDEN members take the next convenient opportunity to review the *deliverables* to ensure these remain aligned to the agreed work plans and programme goals. A review of deliverables would also seem prudent in view of discoveries made within and outside the EDEN project.
  EDEN specific objectives (SP and HITs) are discussed at each steering committee. In-depth discussions are also held during international conferences and workshops which are attended by EDEN scientists. Therefore, we are quite confident that deliverables correspond to state-of-the-art knowledge. However, we asked each SP and HIT leader to revise them at the occasion of the 2008 annual report.

- As the EDEN project moves into its final 3 years and the first risk maps become available, it would seem appropriate to consider in more detail the use to which EDEN data could be put by the scientific community and society. **It is recommended that EDEN hold a stakeholders meeting to establish and agree the most appropriate routes for dissemination of EDEN outputs,** and best ways to present information to meet the demands of what will be a disparate audience.
  Though we did not organise ourselves a stakeholder meeting, we attended many workshops and international conferences organised by European and International public-health agencies (see below). We presented EDEN methods, tools and results and were involved in the working groups elaborating

the conclusions and recommendations. Many EDEN partners were also involved in a so-called V-Borne expertise coordinated by CIRAD, addressing questions asked by ECDC (Stockholm): "Assessment of importance and magnitude of vector-borne diseases in Europe". At this occasion, contacts were considerably strengthened with ECDC, and discussions are still on-going in the frame of the preparation of replies to FP7 calls, or other projects. We also pursued discussions with DG-Sanco and European Space Agency to develop applications combining spatial technologies and EDEN methods and results: disease monitoring and early-warning information systems.

- …The reviewers would like to be reassured that appropriate financial resources are available for safe and effective collection of field samples. **It is recommended that all groups engaged in collection of field samples review their budgets and, where appropriated, management agree to [re-]allocation of addition support.**
  Budget re-allocation was proposed after a comprehensive review of available funds and actual expenses for each EDEN partner. A survey was also carried out, where each partner expressed its specific needs by the end of the project. The conclusions were presented and validated at the September steering-committee. Funds were provided to the beneficiaries together with the third payment. A major reallocation was the appointment of an extra post-doc person year to strengthen the mathematical modelling support provided as an horizontal integration tool to the EDEN sub-projects.

- **The reviewers were particularly impressed by the success of the PhD programme** that has provided unique opportunities for young investigators to develop their own network for scientific exchange and, in its broadest sense, pastoral support. It was notable that students from both large and small institutions felt that their involvement with EDEN provided experience and insight to all aspects of collaborative research that was not available to their peers in their home institutions. **The reviewers encourage the Management and Principal Investigators to continue to build on this success that must be seen as a major contribution to capacity building."**
  We did our best to maintain and develop this dynamic. Besides the annual PhD meeting and because the end of EDEN focuses on disease modelling and integration, we organised with INRA and EHESP (France) an international R_0 workshop in Paris, with world-known invited speakers. A one-day tutorial was also organised at the end of the workshop, and reserved for PhD students. The workshop was attended by 125 people, 44% of them being PhD students. We also tried to develop "upstream" actions, organising master courses and modules using many of the EDEN results and examples.

1.4 Summary of the objectives of the current reporting period

In the three first years of the project priority was given to implementing the first level of integration as summarized in Table 1.2 below to prepare for the identification of disease specific risk factors and models.

Vertical Work-packages	Tasks A (patterns)	Tasks B (processes)
WP1: Landscape, Biotopes and Habitats	Description of the environmental components and habitats (breeding sites, biotopes for vectors, hosts and reservoirs)	Monitoring of major environmental events and their consequences on the cycles components
WP2: Vector Bionomics and Competence	Inventory, host-parasite population dynamics, vector capacity and competence of vector populations	Monitoring introduction, establishment and spread of (exogenous) vectors
WP3: Public Health and Human Activities	Public health demography of the human populations at risk, urbanization	Monitoring of significant population flows between and in countries (rural-urban)
WP4: Animal Reservoirs	Epidemiological inventory of the receptive wild and domestic animal populations	Monitoring migrations (avifauna), movements and transport (livestock) of the animal reservoirs
WP5: Data Management and Modelling	Disease data management and modelling	

Table 1.2 - EDEN's first level integration Vertical Work Package activity matrix

1.4.1 Vertical sub-projects

Whilst in the first year the main focus was on team building, establishment of common protocols, selection field sites, start of field activities, collection of historical data sets, the development of lab tools and the development of a common data archiving system, during the second year the first full season data sets were collected and field activities were extended to cover all the areas under study. This field work routine was further carried out during year three. In addition to the intra sub-project activities the horizontal collaborations towards achieving the second level of EDEN integration, i.e. the generation of disease specific models, were further extended and carried out. Detailed information about specific objectives, activities and teams involved is given in section 2.

Vertical SP	Activity	WP1 Environment	WP2 Vector	WP3 Public Health	WP4 Reservoir	WP5 Data management
TBD	Historical archives	X	X	X	X	X
	Maps & trends	X	X	X	X	X
	Field data collection	X	X	X	X	X
	Lab tools and analysis	NA	X	X	X	NA
	HIT collaborations	Low resolution remote sensing, High resolution remote sensing, Mathematical modelling, Data management				
ROBO	Historical archives	X	NA	X	X	X
	Maps & trends	X	NA	X	X	X
	Field data collection	X	NA	X	X	X
	Lab tools and analyses	NA	NA	X	X	NA
	HIT collaborations	Low resolution remote sensing, High resolution remote sensing, Data management				

LEI 37-48M	Historical archives		X	X	X	X
	Maps & trends	X	X		X	X
	Field data collection	X	X	X	X	X
	Lab tools and analyses	NA	X		X	NA
	HIT collaborations	Low resolution remote sensing, data management, plus environmental change and mathematical modelling				
WNV	Historical archives	X	X	X	X	X
	Maps & trends	X	X	X	X	X
	Field data collection	X	X		X	X
	Lab tools and analyses	NA	X		X	
	HIT collaborations	High resolution remote sensing, Mathematical modeling, Biodiversity, Data management				
MAL	Historical archives	X	X	X	NA	X
	Maps & trends	X	X	X	NA	X
	Field data collection	X	X	X	NA	X
	Lab tools and analyses	NA	X	X	NA	NA
	HIT collaborations	Low resolution remote sensing, High resolution remote sensing, Mathematical modeling, Biodiversity, Data management				
AFR	Historical archives	X	X	X	X	X
	Maps & trends	X	X	X	X	X
	Field data	X	X		X	
	Lab tools and analyses	X	X	X	X	NA
	HIT collaborations	High resolution remote sensing, data management				

Table 1.3 – Summary of work performed by vertical sub-projects

1.4.1.1 Summary of the work performed by TBD

Work Package	Main problems encountered	Solution proposed	Contingency plan in case no solution
WP2	Delay and limited experience amongst some partners in molecular diagnosis of infection in field-collected ticks. Any additional work arising from the analysis of earlier results will therefore be limited.	Laboratory work continued into years 3 and 4. Training visits to Dr Gern's lab, Switzerland (Neuchâtel) to learn Reversed Line Blot techniques for identification of *Borrelia* strains. Analyses to be based on available data.	
WP2	Higher than expected costs and technical challenges for the identification of past blood meals in field-collected ticks.	Data will be available from some partners, probably enough to reveal general patterns.	

The advances made by each partner towards the deliverables are shown in Table TBD-01. It is clear that yet further progress has been made over the past year in filling the remaining gaps in the hard time-series data relevant to the changing epidemiology of tick-borne diseases (TBD), and more standardized data sets have now been prepared and posted on the EDEN web site (marked letter G in the table). Our published studies have established that human social biology and activities are as important as microbial and animal biology and ecology in driving these infectious disease dynamics. These ideas are being adopted by other epidemiologists and wildlife disease ecologists. Most significantly, they are liberating researchers from the straight jacket of blaming all disease emergence on climate change, and giving public health practitioners new tools with which to tackle problems that hitherto might have seemed to be beyond their immediate control. Just as socio-economic development and the deployment of low-tech control devices are apparently bringing down the incidence of malaria in several parts of Africa, so awareness and self-protection (through vaccination or avoidance of high-risk activities) may reduce the incidence of tick-borne diseases amongst residents and travellers in Europe (see publication number EDEN0046 year 3).

Significant advances have been made in our knowledge of the quantitative biology and interactions of all partners (pathogens, vectors, wildlife hosts and humans) within the natural TBD systems in the range of environmental conditions found in Europe. Data on the infection prevalence of some of the less well surveyed emerging pathogens (*Anaplasma phagocytophilum, Babesia* spp) have been collected from many parts of Europe. Similar data for TBE virus and *Borrelia burgdorferi* s.l., recognized as the two most widespread and medically significant vector-borne pathogens in Europe, have been strengthened. Ideas to explain the observed epidemiological heterogeneity can now be tested against real data collected from the field, including physical environmental conditions, seasonal abundance of questing ticks, relative abundance of rodents and large animal hosts, and humans living in a variety of socio-economic circumstances.

Objectives for the next period

Each partner will prepare publications based on the analysis of their own historical and new field and laboratory data. Comparative analyses of data from multiple partners will continue and be undertaken even more actively. To this end, all remaining data will be collated and posted on the EDEN-TBD web site.

Up-dates of epidemiological and environmental data to the end of 2008 will be completed. Partners will remain vigilant for the availability of additional data sources, especially in response to questions that arise during analyses and preparation of publications.

Field data on the following from across Europe will be compared and related to environmental factors: a) tick seasonal dynamics, b) infection prevalence of four pathogens in field-collected ticks, and c) rodent abundance, infection status and tick infestations. Causes underlying the observed patterns will be sought.

Any remaining samples of ticks and rodent sera will be screened for infection, as the need arises following analyses of the results to date, according to available funds.

Detailed analyses of the demographic patterns of TBE cases will strengthen our interpretations of variable risk factors in time and space.

Cryo-preserved rodents trapped in different habitat types associated with the abandonment of agricultural land in Latvia will be screened for TBE virus infection prevalence and the results will be analysed.

The development of an agent-based model will aim to predict geo-specific risk based on human socio-economic and environmental factors. This will incorporate individual human decisions concerning activities that might incur exposure to infected ticks on the basis of economic status, leisure pursuits, vaccination protection, risk awareness and the differential probability of infected ticks in forest habitats.

All partners will consider and actively explore national and international opportunities to build on the significant advances made within EDEN, and to implement our new and deeper understanding of the underlying causes of the dynamics of tick-borne disease systems for public health benefits.

1.4.1.2 Summary of the work performed by ROBO

Work Package	**Main problems encountered**	**Solution proposed**	**Contingency plan in case no solution**
WP1	Some of the Pan-European objectives unclear	More focusing on the GIS background	
WP 5	Mathematical modelling delayed	A new modeller will start in UA, and collaboration with Lyon scientists	

The work on animal reservoirs and viruses has progressed well. We have a clear picture of phylogeography of important hantavirus carrier hosts and their hantaviruses. In addition, research on the MHC-genetics, possibly predisposing rodents to hanta infection has produced interesting results. So far, in discussion of the different human hanta (and other robo disease) dynamics in Europe, we have concentrated on different host dynamics and landscapes. Now we have to seriously consider an additional third factor, that MHC genetics of rodents may affect their susceptibility to robo viruses. MHC genetics are incongruent with traditional mtDNA phylogeographies.

A detailed study on the on S and M segments of Puumala virus (PUUV) in a highly endemic region in Finland went on and showed surprisingly large genetic variation and also reassortment at a local scale We have continued this study by following the abundance and "selection survival" of reassortments from the rodent peak of 2005 down and through the decline in 2005-06 and again to a new peak in 2008-08. We have found the contact zone between N Scandinavian and Eastern PUUV lineages in NW Finland, where all kinds of reassortments have been found.

We have new data on the abundance and distribution of LCMV- type viruses in Europe, and we predict that there are several LCVM- type stains in Europe. We have tried to develop methods to recover the LCMV strains but have not yet been successful. Ecological studies on cowpox have progressed well, and sequencing of cowpox strains has started.

Annual/biannual monitorings are running in several countries. Intensive longitudinal monitoring on host dynamics and robo transmission dynamics continued on three selected regions. In N Italy we completed the first long term data set ever on LCMV dynamics in wild rodents, and showed that masting events impact also LCM dynamics.

In Belgium, small scale climate differences seemed to play a role in PUUV occurrence, vegetation index between years, but the abundance of bank voles does not seem to affect the degree of PUUV seroprevalence further. We found indications for a dilution effect on PUUV prevalence, dependent on the relative proportion of non-host wood mice *Apodemus sylvaticus* in a study site.

We regard the combination of a dilution effect, a possible threshold density that depends on local conditions and a higher fragmentation of suitable bank vole habitat in our study area as plausible explanations for the sparse occurrence of PUUV infection and low prevalence detected. In contrast, in Finland we showed that fragmentation due to intensive forestry does not affect the abundance and ubiquitous occurrence of bank voles and PUUV in northern forest landscapes. Thus, strongly cyclic dynamics and extensive "homogenous" landscapes, and absence of dilution species explain the commonness of PUUV in the North. Due to this, PUUV infections in humans start to appear with only a lag of 2-3 months after rodents start to increase. We have a lot of new info of the role host population structure on robo transmission, prevalences and dynamics in various population subgroups, both in Belgian, Finnish and Italian situations.

We started a joint project with TBE team in Latvia to analyse the impact of habitat change (reforestation and afforestation of former agricultural land) on the occurrence of ROBO and TBE risk.

Experimental work on the kinetics of hanta infection process in rodent hosts showed the major virus output early in the chronic infection. We have subsequently expanded this study to wild rodent populations too see if the results are general, and most of all, if maturation and hormonal changes in the hosts can cause long-term variations in virus shedding. Even if the infection is chronic in rodents, it is essential to know for modelling if the virus shedding is only temporal and short term.

Modelling on masting has produced clear results suggesting that summer temperatures are decisive, and in fact, the outbreak in 2005 in western and central Europe could this way be traced back to the heat wave in 2003. It is important to realize that the processes leading to rodent and robo disease outbreaks are long term processes, because too often, especially in the medical circles without ecological understanding, only the simultaneous climatic events are considered. The Finnish data on rodent cycles and NE local dynamics is being compiled, and the Swedish data on last outbreak has been completed..

The available European human data on HFRS has been compiled, and the diagnostic methods in hantavirus research have been updated and reviewed, including the further development of microarray methods.

The Belgian and Swedish human HFRS data have been and is being analysed together with HR and LR HITs, and Slovenian and Finnish human data are at LR HIT. Finnish local vole and virus data are being analysed at HR HIT. Large human serosamples from Italy and Switzerland have been screened. Ecological niche modelling has been started. The aim of the study is relating environmental features to occurrence of human PUUV infection on a large geographical scale within West/Eastern Europe using new modelling

techniques. After a delay, mathematical modelling of the robo dynamics in Belgium, Finland and Italy has started.
At the annual meeting of year 3 in Brno, all partners and participants in the Robo group hand an extensive review on their work, and several PhD students participated in the PhD meeting. Quite a number of EDEN and EDEN related papers have been published, and ROBO team members have participated in several congresses.

Objectives for the next period

- WP 1.
  - The Pan European maps will be created. What is not necessary at large scale, is often satisfactory at the local scale case studies
  - Updated masting analyses.
  - Possible changes in the winter climate at selected sites (Finland, Sweden)
- WP 3. Updating the human ROBO dynamical and distribution maps.
- WP 4
  - Final analyses of longitudinal transmission studies.
  - Understanding the kinetics of virus shedding in natural rodent populations
  - Immunogenetic analyses based on several genes in relation to mtDNA and PUUV phylogeogrphies
  - LCMV sequencing and strain variation recovered
  - Analysis of cowpox dynamics and possible strain variations
  - Studies on landscape change impacts completed
- WP 5
  - Mathematical modeling on Belgian and Finnish PUUV data
  - LR analyses on several countries updated and completed
  - HR analyses on Finnish and Belgian data completed
  - Ecological niche analyses completed
  - Multipathogen risk maps produced (together with other SPs)

1.4.1.3 Summary of the work performed by LEI

Work Package	Main problems encountered	Solution proposed	Contingency plan in case no solution
WP1	Partners UB 40 (Barcelona with Granada associate) and ISS 12 lack a local geographer and are unable to produce and/or use datalayers, despite training workshops using partner LSHTM 36 masters' practical exercises.	ARC GIS training is being undertaken in Madrid by partner UB 40. Partner ISS 12 to allow spatial analysis to be carried out by partner LSHTM 36.	Jon Cox (LSHTM 36) to visit Barcelona and Rome if skills established locally (cf. statistical help visit to Madrid in WP5)

WP2	Partners SZIE 37 and UoC 40 have not carried out standardized sampling of sandflies suitable for statistical analysis	Partner SZIE 37 to submit a sampling plan to SP co-ordinator [5th year sampling justified by novel records from Hungary]; partner UoC 40 to stop EDEN sandfly sampling	No funds for further sampling of sandflies
WP2	Partner NHM 34 has not completed spatial analysis of all historical sandfly records	Partner UB 40 to send historical sandfly records of northern and southern Spain	Analysis excludes most of Spain
WP3	Absence of conclusion on European initiative to standardize national surveillance systems for human leishmaniasis	SP co-ordinator (NHM 34) to propose co-authoring ECDC publication with co-ordinator of LeishRisk project (EU FP6) and partner UM1 39	Proposal to be accepted or finally rejected at WorldLeish4 congress in India, February 2009
WP4	Absence of conclusion on European initiative to standardize national surveillance systems for canine leishmaniasis	SP co-ordinator (NHM 34) to propose co-authoring ECDC publication with co-ordinator of LeishRisk project (EU FP6) and partner UM1 39	Initiative to be accepted or finally rejected at WorldLeish4 congress in India, February 2009
WP4	Partner SZIE 37 has not carried out standardized sampling of canine leishmaniasis suitable for statistical analysis	Partner SZIE 37 to submit a sampling plan to SP co-ordinator [5th year sampling justified by novel records from Hungary]	No funds for further sampling of Canine leishmaniasis
WP5	Many partners lack a local statistician and are unable to perform basic spatial analyses, despite training workshops using partner LSHTM 36 masters' practical exercises.	Jon Cox (LSHTM 36) and SP co-ordinator to visit Madrid (Partner CNM 9 visited 1-3 December 2008), Barcelona and Rome, if skills established locally	Partners to allow spatial analysis to be carried out by partner LSHTM 36.
WP5	No roadmap for delivering R_0 model for canine leishmaniasis in France, because of unavailability of geographer in collaborating HITs	Hans Heesterbeek & Nienke Hartemink (Modelling HIT) to update David Rogers (Low Resolution HIT), Eric Lambin (Environ HIT) & Paul Ready (LEI data provided January 2008 & September 2008)	LEI-HITS collaboration to terminate
WP5	Failure to publish or to provide provisional details of authors, title and journal for each publication in preparation	All LEI partners to send to EDEN Steering Committee a list of planned publications, including provisional details of authors, title and journal	No further EDEN funds
WP1-5	Long-term illness (cancer) of PI of partner LSHTM 36, supervising GIS and statistical analyses	Research assistant recruited for 6 months (from October 2008) after obtaining approval of Steering Committee	Funding of research assistant to be extended for at least a further 3 months

Good progress was made with completing field surveys of environments (WP1), the sandfly vectors of *Leishmania infantum* (WP2), and canine leishmaniasis (WP4) by the LEI partners: CNM 9 (central Spain), UB 38 (north-eastern and southern Spain), UM1 39 and NHM 34 (France), ISS 12 (northern and central Italy), SZIE 37 (southern Hungary), UoC 40 (Attica, Greece) and EUMS 35 (western Turkey). Concerning planning and data analysis (WP5), sub-project workshops were held at the EDEN AGM in Brno (month 39) and in Granada (month 44). Collaborations with the HITS were continued, with the aim of producing Ro biological models (with the Mathematical Modelling, the HR Environmental Change and Low Resolution modelling HITs). Data analysis and modelling were delayed by illness affecting some partners and the difficulty of recruiting a replacement researcher.

Objectives for the next period

In M48-60, the focus will be on data processing and analysis, in order to complete the project by producing regional and Europe-wide spatial models for at least the main components of widespread leishmaniasis transmission cycles, particularly the regional sandfly vectors (WP2) and the canine reservoir hosts (WP4) of *Leishmania infantum*.

1.4.1.4 Summary of the work performed by WNV

Work Package	Main problems encountered	Solution proposed	Contingency plan in case no solution
WP2	Mosquito infection studies have not begun because of lack of expertise after departure of arbovirologist in 2006	New technician now fairly confident in level P3 techniques; new post-doc will be recruited for one year. Data from current study (on vertical transmission of yellow fever virus in Aedes aegypti) will contribute to study of WNV in Culex spp. identified as putative vectors.	Unlikely to be necessary
WP3	Ethics of human serosurvey too complex to achieve by partner.	Abandon work-package	Serosurveys of horses will have to suffice as sentinel indicator
WP5	Teams reluctant to share some forms of data	Wait till primary publications have been submitted, then encourage publications with authorship of all teams. The latter will fulfil the primary objective of the whole sub-project: learning through comparison between study areas	

Objectives for the next period

The emphasis in this period must be on data analysis and production of manuscripts. All teams have made progress on this front, some to an advanced stage, but there remains much to be done. Most teams are eager to publish their own results before sharing data with other teams. I see little way around this, but will emphasize the need for inter-team publications when we meet in Marrakesh. This will be my main role for the rest of the project though I will continue to guide the entomological study in the Danube Delta, which has given such interesting results in the past season. I will assist members who have difficulty in writing in English to produce manuscripts that are suitable for submission to high profile journals. Lastly, I am in the process of recruiting an experienced arbovirologist to work with my *Ingenieur* (senior technician) on vertical transmission; the latter has now learned basic techniques that are essential for this work. Recent results in the Danube Delta (NIRDMIC and DDNI) provide evidence that this may occur at a high frequency, at least in some areas.

1.4.1.5 Summary of the work performed by MAL

Work Package	**Main problems encountered**	**Solution proposed**	**Contingency plan in case no solution**
WP1: Landscape, Biotopes and Habitats	Several teams are late in publishing and disseminating their ecological maps	Most of these partners already have data. They must spend time in putting them in a proper format for dissemination and for developing environmental risk maps	WP1: Landscape, Biotopes and Habitats
WP2: Vector Bionomics and Competence	The main problem was that our Nijmegen subcontract has already finished his job (i.e. the number of tests initially planed), while some mosquito population remain to be tested	Involving an other partner. Pasteur Institute in Paris has kindly accepted to test mosquitoes from Italy and Corsica. Pasteur Institute agrees to do more in 2009	WP2: Vector Bionomics and Competence
WP3: Public Health and Human Activities	All teams have made sociologic surveys or have worked on imported malaria. However quality of data highly depends on teams.	The SP leader will discus with the teams during 2009 annual meeting	WP3: Public Health and Human Activities

In 2008, all the teams focused on completing the field data bases, which now cover 4 project years, the development of models of mosquito distribution and risk based on these data sets, and on the publication of the obtained results. Mosquito parameters have been recorded in all regions from the project. Most of the partners report the development of a GIS including environmental data. However, such tools remain to be adopted by some partners. Molecular and population genetics studies have been conducted on Anopheles atroparvus, An. labranchiae and An. hyrcanus through the EDEN MAL collaborative network, and results will be presented during the 2009 Annual meeting. Vector competence has been assessed by experimental transmission of an African Plasmodium falciparum strain, in Nijmegen. Almost all partners have sent mosquito populations to Nijmegen to be tested. All species but An. hyrcanus were able to replicate P. falciparum, however rates of infection were generally low compared to highly susceptible control strain, and depend of species and rearing conditions. Nijmegen subcontract partner has already tested all the mosquitoes as initially planed. Some additional mosquito populations remain to be tested: i.e. Romania, Algeria, Morocco, and Spain. Since these cannot be tested in Nijmegen a satisfactory alternative solution was found at the Pasteur Institute in Paris. In 2008 An. labranchiae from Corsica en Italy was tested by IP, and it is agreed to test more in 2009. The studies related to disease, human health, human migration and imported/ autochthonous malaria made good progress during year 3 and 4 and are almost finished in some countries. Epidemiological R0 models remain under development (finished in France, initiated in Spain, Algeria, Turkey, Morocco), as well as multi agent models (finished in France, initiated in Portugal). Ideally these approaches are developed in collaboration with the relevant HITs (horizontal teams). However HIT don't have the capacity to collaborate with all (the 40 or so) vertical teams, and data collected by these vertical teams are not always robust enough for developing models. The malaria networks are fully operational. Several scientists and students have visited other partners. Many posters and communications related to EDEN MAL have been presented during national and international meetings.

Many scientific papers related to EDEN MAL have been published or submitted during year 4. Two EDEN MAL PhD thesis have been finalized in 2008.

Objectives for the next period

Main objectives are to complete data when necessary, and to publish data in scientific journals. AS much as possible priority will be given to integrative papers using horizontal tools such as GIS, remote sensing and modeling. This will be achieved through mobilizing existing MAL partner competences, or in collaboration with or with guidance from the horizontal teams.

1.4.1.6 Summary of the work performed by AFR

Work Package	Main problems encountered	Solution proposed	Contingency plan in case no solution
WP1	Landscape description was not updated for this reporting period.	Will be discussed with landscape specialists at the AGM (AL Tran – CIRAD and E Lambin – UCL).	
WP2 and WP3	IPD has colonised several candidate vectors species in their laboratory however competence and capacity test not yet achieved because of security problems.	IPD should find alternative ways to finalise the security works in its insectarium.	Establish a sub-contract between IPD and another institution to do the work, like was done in the Malaria sub-project.
WP4	Animal mouvments have been studied in Senegal but not in Mauritania or even in Morocco.	Find a solution during AGM in Marrakech, and possibly visit the Moroccan veterinary services to get their support.	
	Serum collected from migratory and resident birds have been analysed and results generated. However due to misinderstanding betwenne IRD and Pasteur these results are still not released for general use.	The condition required by IPD is that these results will be handed over to a scientist at IRD who could be in a position to discuss them for a joint publication. This matter will be discussed again during the annual meeting because despite numbers attempts made it remains one of the main problems of the platform.	Find a solution with another partner who will be able to analyse the data. CIRAD may be an alternative. Will be discussed with F Monicat during the AGM.
WP5	CIRAD data management scientist has been moved from Dakar.	Work may continue without physical presence in Dakar. Will be discussed with CIRAD and HITs during the AGM.	

The EDEN Africa platform has consolidated the results obtained so far, e.g. the collection of field data on candidate vectors using a variety of trapping methods. This allowed the discrimination between host-attracted mosquito species and species actually biting the hosts, using horse-baited and chicken-baited traps. RVF surveillance was conducted on the border between Senegal Mali and Mauritania. Among the 240

ruminant sera which were analysed, a single (weakly) IgM-positive serum was detected. The risk period for horse West-Nile virus seroconversion was identified: most negative horses became positive between October and January. Analyses on wild and resident bird sera have been conducted and new results are available but not disseminated yet.
Studies on potential RVF and WNF vectors bionomics have continued. Captured mosquitoes were dispatched in monospecific samples for virus research; however, in addition to analyses previously reported, no virus was isolated from these mosquito batches.
Rearing of vector candidates *Aedes vexans* and *Culex poicilipes* is now routinely achieved. However, for practical and administrative reasons, the study of vector competence of these mosquitoes has been postponed until satisfactory biosafety conditions are met.

EDEN Africa platform is still facing two main problems:

- difficulties between IRD (partner n°13) and Institut Pasteur de Dakar (IPD, partner n°48) to find an agreement as to whom hands over results on analyses made by IPD on the resident and migratory birds sera samples collected by IRD, and on the other hand the difficulties in the data management within the platform.
- Data sharing is a recurrent shortcoming of the platform complicating coordination during this reporting period.

Objectives for the next period

During the next reporting period, a major effort will be done by all the partners of the African platform to analyze and model the existing data, and to write scientific papers.

1.4.2 Horizontal integration teams

The aim of the horizontal integration teams (HIT) is to provide shared state of the art data and methods in order to enhance scientific and technical integration in EDEN and provide all partners with additional inputs to reach the objectives of EDEN. In this third reporting period the main tasks of the horizontal integration teams was to establish and make available through the EDEN website common data bases of spatial data sets; provide processed low resolution remote sensing data to prepare for disease modelling, provide high resolution remote sensing data matching field study sites; contribute to the EDEN strategy document; and to further implement horizontal integrative research collaborations with the vertical subprojects.
Major progress has been made on all fronts. Details of achievements and partners involved are given in section 2. The main achievements of this reporting period are per HIT:

1.4.2.1 Summary of the work performed by "Data management"

Main problems encountered	Solution proposed	Contingency plan in case no solution
No specific problems	N/A	N/A

a) Maintenance and Servicing existing products and collaborators
Both the main data site and it associated EDEN PhD site continue to expand in terms of membership, search engine visibility, and use. This requires significant resources to maintain and service. A new server was purchased and installed in June 2008, and the old one reconfigured as a backup. The site is thus now both faster, and more reliable. Combined usage regularly exceeds 150 unique visitors a day which is remarkable for a specialist technical site. A high proportion of these visitors are from outside the EDEN community, which means that the site is significantly enhancing EDEN's visibility and profile to the 'outside world'. The success of the EDEN PhD Site in particular has significantly added to the training element of the DMT activities

b) Expanding provision of standardised georeferenced datasets to Data site Members

A wide range of new datasets have been provided for the users, many of which are in the throes of detailed analyses of the data collected during EDEN's early years. These include: EU wide trees species distributions (specifically sourced for ROBO and TBD subproject members); remotely sensed indices of vegetation phenology; revised Administrative Unit boundaries at several levels for all EDEN countries; Climate change projections for temperature and rainfall from a number of sources, one in collaboration with the ECDC Funded Vborne project; new high resolution land use land cover layers ; Mammal and Avian Diversity mapping tools and outputs. There are now nearly 900 datasets in 90 data groups in 20 categories.

c) Preparation of final outputs due in 2010

Subproject data continues to expand at only a modest pace – with new information for TBD and LEISH subprojects. It has become very evident that sharing raw project data (particularly disease related information) through a central site is acceptable to only the closest collaborators. As a result the emphasis of the subproject data sharing has been moved from the exchange of sensitive data between relatively few researchers to the widest possible dissemination of what data have been acquired, from where, using what methods, and by whom, alongside a summary of any results that have been published. The intention is to describe and draw attention to the researchers and their data resources, without endangering their intellectual property rights, so that potential collaborators or indeed funding bodies can identify who best to approach for certain kinds of information and expertise. This searchable, georeferenced and mapped database is being compiled as one of the main components of the EDEN Information System that is a primary output of year 5. A number of other elements are being designed and constructed:

d) Preparing for the continuation of the data archive facility beyond the end of the project

Efforts have been in train for much of 2008 to find ways to extend the life of the EDEN Datasite beyond the end of the project, so that its content continues to be available to the network of researchers that has been developed by the EDEN approach. A number of possibilities are under discussion including the adoption of the site by the European Centre of Disease Prevention and Control in Stockholm. This would include the active advisory involvement of the ECDC in determining the details of the final DMT outputs due in 12 months time

<u>Objectives for the next period</u>

For the final twelve months the DMT will have the following six primary objectives:

a) Maintenance and support of the current websites and data archives. Given the increasing complexity of these products, this is likely to require a substantial proportion of the available resources.

b) Identification and addition of data to the website. This will include public domain data, as available, and EDEN data as provided by the Sub-Projects

c) Provision of technical assistance and advice for GIS and data management to EDEN partners as requested. Given the level and number of requests received to date, it is not envisaged that training courses will be needed, and the majority of assistance will most probably be implemented via email replies to specific questions and queries.

d) Epi-GIS distance learning tool.

e) Production of the EDEN Information System

    a. EDEN scientific data spatial information system (continued as started)

    b. EDEN ecosystem at risk spatial information system: The primary aim of EDEN was to: "Identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change, which can influence the spatial and temporal distribution and dynamics of pathogenic agents." Therefore the objective is to development queryable DVD based GIS tool which enables to query a spatial data archive of selected data to provide answers to questions related to the original EDEN aim. The system will run in two modes: (a) Pre-prepared query outputs with ready to use analysis outputs and (b) Free queries and analysis by advanced users either using the included data sets. Whilst the knowhow to develop such a tool is available within the DMT team, its development and implementation will greatly depend on inputs provided by the SP leaders.

Negotiations for the continuation of the EDEN data archive in a permanent location.

1.4.2.2 Summary of the work performed by "HRRemote sensing"

Main problems encountered	Solution proposed	Contingency plan in case no solution
No specific problems	N/A	N/A

Collaborations with several teams of vertical sub-project continued. Following these, images and data were processed and analysed. Statistical analyses were performed and models developed. Some of the results obtained have been published in joint publications.

Objectives for the next period

Complete empirical studies with vertical sub-project and integrate findings across all HITs.

1.4.2.3 Summary of the work performed by "LRRemote sensing"

Main problems encountered	Solution proposed	Contingency plan in case no solution
Post-doc position filled (after three attempts!) only in October	NA	NA

Collaborations with several teams of vertical sub-project continued. The continuous MODIS datastream is being captured and processed as imagery is release by NASA, giving us a complete time series of environmental data for Europe from 2001-date. Statistical models of various sorts are being refined. Integration with other HITs and SPs has started.

Objectives for the next period

The main objective is to integrate HIT and SP activities through demonstration analyses of SP data collected before and during the life-time of EDEN. We will quickly eliminate the backlog of analyses resulting from the absence of an LRRS post-doc for much of the present reporting period. We need carefully to consider the future of EDEN's many activities beyond the end of EDEN. The EDEN community is a valuable resource for the whole of Europe and we must do our best to sustain its collective skills into the uncertain future.

1.4.2.4 Summary of the work performed by "Mat Mod"

Main problems encountered	Solution proposed	Contingency plan in case no solution
Delay in obtaining additional data from the Leishmaniasis team due to illness of main data expert in that team	The situation has now been resolved (at the end of the reporting period) so in the next (5th) reporting period the joint work can be finalised	

Worked with malaria vertical team and with Leishmania vertical team in this accounting period + worked with LR and HR Hit teams. Produced one paper for on methodology. Solved problems with Leishmania data.

Objectives for the next period

Main objectives: 1. Prepare manuscript on methodology integrating LR-Hit and Mod-Hit (BTV as case study) for new journal; 2. Finish work on Leishmania for South of France, integrating LR-Hit, HR-Hit and Mod-Hit for the first time with a vertical team; 3. Finish paper on sensitivity analysis of R0 using Tick-borne diseases as case study; 4. Finish PhD thesis; 5. Assist all vertical teams with modelling and R0-risk mapping (work with the malaria team has already started).

1.4.2.5 Summary of the work performed by "BioDiv"

Main problems encountered	Solution proposed	Contingency plan in case no solution
One of the main puzzling problems encountered by HIT BioDiv was the access to disease data for comparison with epidemiological modelling. The main reason seen is the time lag between HIT BioDiv PhD's work starting date and core research work within EDEN Collaboration with EID' Gregory L'Ambert partner on Culex spp. population dynamics in the Camargue area has been post-poned, and it will start next January 2009	Solution was to use US data for West Nile Virus fever to extrapolate to European conditions like in the paper Disentangling the effects of intrinsic susceptibility and ecological factors for epidemic patterns. The case of West Nile Fever.	

Objectives for the next period

The main objective for the next period is the writing of three papers and training of G. L'Ambert *PhD* student to population dynamics principles. One paper will summarize new findings obtained within EDEN on the linkages between biodiversity and infectious diseases. The targeted journal is *Trends in Ecology and Evolution*, in which we will synthetize the pros and cons of biological diversity in prevailing or not against disease invasion (see the recent book by Ostfeld *and coll.* 2008). A second paper in preparation deals with a comparative approach between host (reservoir/vector) life-history traits and capacity/competence in disease transmission across host species using West Nile virus and their bird reservoir species. In this work, we are focusing on some proxies, *ie.* biological and ecological traits, that could be good indicators of host capacity and competence in the transmission of West Nile Virus disease. A third paper in collaboration with G. L'Ambert (EID), D. Fontenille (SP MAL) and A. Tran (HIT HRRS) will be on the population dynamics and seasonality of *Culex pipiens* in the Camargue area as a potential vector of West Nile virus transmission.

1.4.3 Management, training and dissemination

The main objective of the central coordination team was to provide all necessary back-up to enable smooth functioning of the project; administrative and financial guidance for partners, distribution of the EC contribution, application of guidelines for management and report, problem solving, as well as pan-EDEN team building, facilitation of communication between project teams, promotion of proper understanding of scientific fields and objectives and organizing the dissemination of EDEN results.

1.4.3.1 Summary of the work performed by "Coordination"

Main problems encountered	Solution proposed	Contingency plan in case no solution
Some residual delays related to field data collection.	Whilst these may affect results in some specific field sites (mainly EDEN AFR) no major impact on overall EDEN results are anticipated. Nevertheless during the Marakesh AGM coordination will make a more detailed inventory of these data gaps.	Focus should be on data analysis and publications; in case a major gap is identified teams will be urged to collect remaining data.
Delays related to data analysis which may impact the integrated analysis of EDEN data:		
1. Additional LEI data analyst	Due to severe illness of the main data analyst of the LEI SP this part of the work was delayed. Funds have been reallocated to hire additional analysis time enabling integrated data analysis.	Funds are available and person is operational, no further problems anticipated.
2. Recruitement LRRS postdoc	This position was open since the start of the project but could not be filled due to recrutement problems. A person was now recruited.	Funds are available and person is operational, no further problems anticipated.
3. Complementary MatMod postdoc	EDEN funds were reallocated to enable the further recrutement of the MatMod PhD on a one year postdoc as a support to all sub projects.	Funds have been reallocated and person is identified, no problems anticipated

During the 4th reporting period work focussed on completing field activities as well as strengthening data analysis and modelling. Several problems were overcome, such as the recruitment of short-term positions and postdocs: (i) a complementary SP Leishmaniasis position, to prepare data for modelling, (ii) the delayed recruitment of the HIT LRRS postdoc, to model the impact of environmental changes on disease emergence, and (iii) a complementary HIT Mathematical modelling postdoc, to complete the work started with the PhD thesis of Nienke Hartemink. The two complementary posts were made possible by internal reassignment of EDEN funds. Whilst all EDEN data bases are not yet completed and available as planned (mainly EDEN AFR and LEI) contingency plans have been activated to alleviate this. The overall scientific achievement of the project is excellent with ca. 130 publications in the peer-review system as per Dec. 2008 (75 in Dec. 2007).

The "Malaria" and "Tick-borne diseases" sub-project have completed very comprehensive studies on several of their field sites, and elaborated different types of predictive risk models. The "Rodent-borne diseases" sub-project has provided the scientific community with an impressive set of high-level publications in all their activity domains, and is now engaged in very promising modelling work. A critical phase has been overcome by the "Leishmaniasis" sub-project. Though few publications were released up to now, an ambitious collaboration has begun with all the horizontal integration teams, to also produce leishmaniasis and vectors distribution and risk maps.

The last year of the project will focus on encouraging partners to publish all their results, and producing the expected integrative papers to reach the initial goals of EDEN: elaboration of generic models for the emergence of vector-borne diseases in Europe, and categorisation of European ecosystems according to the risk of vector-borne disease emergence. The final event organised by EDEN will be an international conference on the impact of environmental changes on emerging, vector-borne diseases. It will be held in Montpellier (France), in May 2010. The proceedings of this conference will be the final version of the EDEN strategy document which summarizes the changes achieved in the state of the art.

A lot of efforts were devoted to EDEN dissemination during 2008. EDEN scientists and members of the steering committee were heavily mobilized to attend a variety of international meetings and workshops, in particular with public-health agencies such as WHO and ECDC, and in world conferences such as the United Nations Climate Change Conference in Poznan, Poland. Several master courses were developed and given to students of European and international origins (with a special attention for Africa). International workshops were also organised where a large floor was given to EDEN scientists, for presenting their methods, tools and results. Skills of EDEN partners were utilized to conduct an expert consultation on behalf of ECDC (European Centre for Disease Prevention and Control, Stockholm): "Assessment of magnitude and importance of vector-borne diseases in Europe". Discussions were pursued with ESA and DG-Sanco to develop applications combining spatial technologies and EDEN methods and results to disease monitoring and early warning systems. FP7 projects began, involving EDEN scientists: e.g., ArboZooNet, a coordination action for the diagnosis and surveillance of several vector-borne diseases. We are also actively involved in the preparation of replies to released FP7 calls, and were consulted as "think tank" to prepare future calls.

In conclusion, studies on specific disease patterns and processes (health-environment research) conducted during the first half of the project have already enabled the development of integrative disease transmission models. In some cases (e.g., malaria in Camargue, France), these models may be readily translated into tools to assist decision support and to feed dissemination. We have good hope that this type of result will be available for wider areas and other diseases before the end of EDEN. Therefore, reaching the ultimate objectives of EDEN is realistic within the (extended) time-frame: (i) develop generic disease models – including risk maps, and (ii) propose tools and scenarios for disease monitoring and early warning systems.

Objectives for the next period

- Organisation of the annual meeting in Marrakech, including a steering committee meeting.
- Organisation of two other SC meetings in June (UK) and December (Brussels) 2009. Depending on available resources, a final SCM may be organized in April/March 2010. The main goals of these SC meetings will be to facilitate the integration of the specific results obtained to date, and the production of generic models of vector-borne disease emergence, and the categorization of ecosystems at high risk of vector-borne disease emergence.
- During the AGM and the SC meetings, a particular attention will be paid to the organisation of an international conference at the end of EDEN (May 2010), to present and share the most prominent and integrative results to the international scientific community.
- Finalisation of the periodic activity report (4th 12-month period), including the periodic management report.
- Regular management of the consortium, including relationships with the European commission.

1.5 Time frame for EDEN key-activities

Based on the gained experience the following timeframe for EDEN key activities is proposed for the full duration of the project (Fig 1.3). It includes the 7-month no-cost extension which was requested to the European commission, as proposed by the external reviewers after 2008 annual report and meeting.

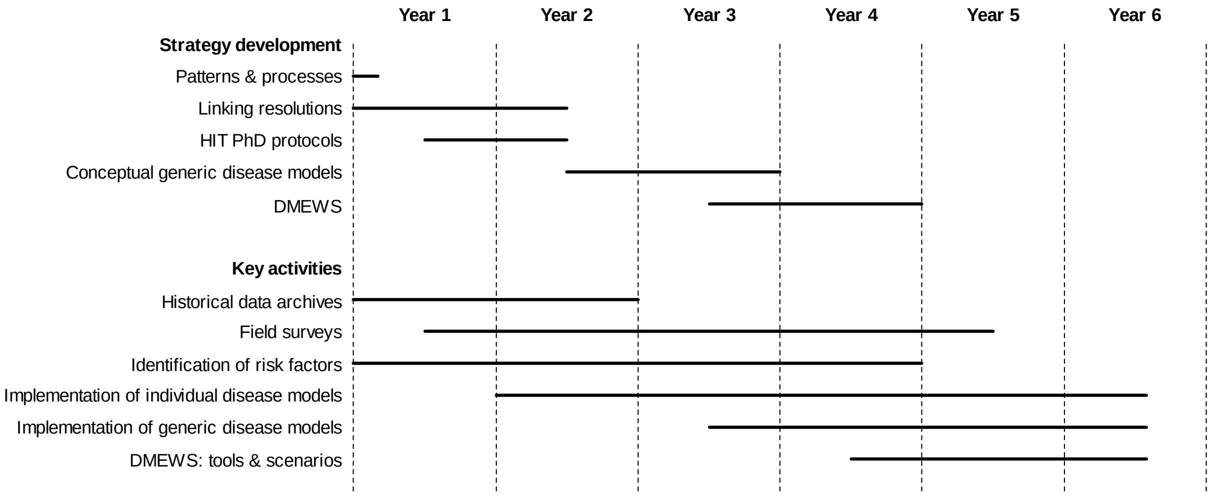


Fig 1.3 – EDEN key integration activities

The title "strategy development" refers to the different parts of the strategy document which will be written during the lifetime of the project. It includes the development of concepts and methods requested in order to achieve the objectives of EDEN:

- Identification of diseases patterns (where, when?) and processes (how?)
- Linkage between scales (both temporal and spatial),
- Building of specific competences (PhD for horizontal activities),
- Design and structure of the general disease models: metadata, indicators, analyse machinery…
- Concept, foundation and design of the Diseases Monitoring & Early Warning System.

Consequently, key-activities refer to multi-team integration activities include:

- The record of available historical archives (*"describing the past to understand the present and predict the future"*)
- Fields activities for the understanding of the epidemiology of selected diseases
- Description of risk indicators and development of diseases specific models
- DMEWS: requirements of such systems and assessing to what extend EDEN can contribute

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Section 2 – Workpackage progress of the period

Workpackages TBD 1 to TBD 5: Tick borne diseases

Deliverable review

Almost all the original deliverables stand, although there has been considerable delay in screening the field-collected ticks for infection with the range of tick-borne pathogens due to the payment delay. The only significant change is that not all partners will be able to complete the analysis of blood meal identity due to lack of expertise in this particularly demanding task and limited funds and time.

Work package	Deliverable number	Deliverables description	Programmed delivery month	Partners - progress/problems															
				ZOOX	CEA	LMU	SAS	IVB	PHA	TAI	CCDPC	DIDN	OEK	VMRI	IMI	NRDMIC	CNM	NEIKER	
				UK	Italy	Germ	Slovak	Czech	Latvia	Estonia	Lith.	Poland	Hung.	Hung.	Slovenia	Romania	Spain	Spain	Switz
				4	18	20	21	11	26	22	27	19	25	47	23	43	9	24	
WP1	TBD01	Climatic data 1970-present	18		G	G	G	G	G	G	G	G	E		G	G		G	E
WP1	TBD02	Land cover/use change	18				D		E	E	E	E	(E)/2		E	E		(E)/2	
WP2	TBD03	Tick populations 1970-present	12		E/2	D/2	E/2	E	E	E	E/2	A/2		(E)/2	E/2	(E)/2		E/2	A/2
WP2	TBD04	Field sampling sites & protocols	15		G	G	G	G	G	G	G	G		G	G	G		G	F
WP2	TBD15	Monthly counts of ticks at field sites	48		G	G	G	G	G	G	G	G		G	G	G		G	G
WP2	TBD05	Molecular diagnostic techniques	18			G	F								G		F	F	F
WP2	TBD16	Preliminary data on infection in ticks	30		E	E	E	E	E	E	E	E		E	E	E		E	E
WP2	TBD17	Preliminary blood meal identification	48		(A)	C	(A)	(A)	(A)	(A)	(A)	(A)		(A)	(A)	(A)		(A)	F
WP3	TBD06	Local TBD incidence 1970-present	12		G	G	G	G	G	G	G	G	G		G	(E)/2	E		G
WP3	TBD07	Public health info 1970-present	12		(E)/2	(E)	E/2	G	G	G	G	G	G		G	(E)/2	(E)		C
WP3	TBD08	Socio-economic patterns from 1970	18		G	G	G	G	G	G	G	G	G		G	G	G		C
WP4	TBD10	Rodent trapping sites & grids	15		D		F												
WP4	TBD18	Population dynamics, rodents & ticks	48		E		E												
WP4	TBD11	Wildlife host abundance 1970-present	18		G	G	G	G	G	G	G	G	G		G	E		G	G
WP4	TBD12	Livestock abundance 1970-present	18		(E)/2	G	G	G	G	G	G	G	G		G	G		G	G
WP5	TBD13	Collated geo-ref data-base	48	G															
WP5	TBD14	Preliminary analysis, spatial	18	G															
WP5	TBD19	Time series analysis	48	G															

Action

(A)	Unlikely to be achieved
B	Sources identified, requests submitted
C	In progress
D	Data collected, not sent to Oxford
E	Data files sent to Oxford
(E)	Text descriptions sent, data do not exist
F	Well established techniques in progress
G	**Database/information compiled and posted on EDEN website**

Problems

0	Finding sources
1	Ongoing data (paper archived) input
2	Few/incomplete data/only monthly or annual means
3	Delay in data delivery from archive
4	Ongoing file cleaning, revision or seeking for missing items

Table TBD-01. Deliverable status per TBD

Executive summary

WP1: Climatological patterns: exceptional weather during 2006-2007 was characterized in relation to the spike in TBE incidence in 2006 in several, but not all, countries.

WP1: Land cover, land use and land tenure were all shown to be significant factors in determining differential probability of TBE cases in rural parishes in Latvia.

WP2: Tick sampling in year 3 was completed at 81 sites. Data from years 1 and 2 from eight countries were used to show that annual and seasonal variation in TBE incidence is not directly related to tick abundance.

WP2: Tick infection prevalence: ticks from 11 countries were screened for infection with TBE virus, *Borrelia burgdorferi* s.l. strains, *Anaplasma phagocytophilum* and *Babesia* spp., with the material from the remaining two countries partially processed. No analysis of the results has yet been conducted.

WP2: Blood meal identity in field-collected ticks: procedures are under way in at least two laboratories.

WP3: The spike in TBE incidence in 2006 was documented in detail.

WP3: The upsurge in TBE incidence in the Baltic States in the early 1990s was shown to be independent of changes in public health practices.

WP4: Rodent trapping was carried out in Latvia in collaboration with EDEN-ROBO to explore the colonization of abandoned agricultural land by the transmission hosts of TBE virus and rodent-borne viruses. Preserved rodents have yet to be screened for TBE virus infection prevalence and the results have yet to be analysed.

WP4: Rodents were trapped for the third year at two sites in Slovakia to explore the impacts of different biotic and abiotic conditions on tick infestations and infection prevalence with TBE virus. Rodents trapped in Italy were screened for TBE virus positive serology.

WP5: GIS preparation and analysis: environmental conditions in northern Italy were analysed and presented spatially.

WP5: Environmental and sociological explanations for epidemiology: the spike in TBE incidence in 2006 in several, but not all, countries was apparently due more to human activities than to tick population dynamics in response to weather.

WP5: TBE risk mapping: the shifting distribution of TBE cases from 1970 to 2004 in Hungary was analysed in collaboration with HIT-LRRS to search for environmental correlates.

Publications

The following PhD student has completed her thesis at least partially using EDEN data:

Anne Kupca, LMU20, Seasonality of *Ixodes ricinus,* and TBD prevalence in Bavaria.

The following PhD students are currently conducting research using EDEN data:

Johannes Heyl, LMU20, Interactions between reservoirs, ticks and pathogens in Saalfeld-Rudolstadt.
Markus Neteler, CEA-FEM18, Remote sensing and prediction of tick-borne disease risk.
Giovani Carpi, CEA-FEM18, Molecular epidemiology of tick borne pathogens in northern Italy.
Ivana Ciglerova, SAS21, Altitudinal distribution of ticks and tick-borne pathogens in Slovakia.
Irina Lucenko, PHA26, Lyme disease epidemiology in Latvia.
Olga Katargina, TAI22, Emerging tick-borne pathogens in Estonia: distribution and genetic characterization
Kériné Balogh Zsuzsanna, OEK25,
Natasa Knap, IMI23, Influence of specific abiotic and biotic factors on transmission of tick-borne pathogens.
Ana Saksida, IMI23, Host immune mechanisms and viral load in the pathogenesis of hemorrhagic fevers caused by Bunyaviruses.
Katja Strasek, IMI23, Molecular characterisation of Anaplasma phagocytophilum in Slovenia.
Miša Korva, IMI23, Prevalence and molecular characterization of TBE Virus in Ixodes ricinus Ticks Collected in Slovenia
Claudia Colpan, NIRDMIC43, Vectors for Borrelia burgdorferi in Romania.
Jesus Barandika, NEIKER24, Studies on ticks and tick-borne pathogens in Northern Spain.
Caroline Burri, University of Neuchatel, Switzerland, Role of rodents in TBE circulation in endemic and non-endemic areas, and identification of new or suspected TBE foci by detection of TBE virus in ticks.

The following scientific documents have been published by EDEN-TBD team members. Those marked by an EDEN publication number were officially validated by the EDEN Steering Committee, those marked by EDENXXX were supported at least partially by EDEN funds but were submitted for validation to the EDEN SC after publication (no EDEN number included in final paper).

Publications of EDEN-TBD (with an EDEN number)

RANDOLPH, S.E. 2008. Tick-borne encephalitis incidence in central and Eastern Europe: consequences of political transition. *Microbes and Infection* **10**, 209-216. EDEN0073

RANDOLPH, S.E. 2008. Perspectives on climate change impacts on infectious diseases. *Ecology* (in press). EDEN0090

BARANDIKA, J.F., HURTADO, A., GARCIA-SANMARTIN, J., JUSTE, R.A., ANDA, P. and GARCIA-PEREZ, A.L. 2008. Prevalence of tick-borne zoonotic bacteria in questing adult ticks from northern Spain. *Vector-borne and Zoonotic Diseases*, (in press). EDEN0099

RANDOLPH, S.E. 2008. Tick-borne encephalitis virus, ticks and humans: short- and long-term dynamics. *Current Opinion in Infectious Diseases* **21**, 462-467. EDEN0100

ŠUMILO, D., BORMANE, A., VASILENKO, V., GOLOVLJOVA, I., ASOKLIENE, L., ZYGUTIENE, M. and RANDOLPH, S.E. 2008. Upsurge of tick-borne encephalitis in Baltic States at political transition, independent of changing public health practices *Clinical Microbiology and Infection* (in press). EDEN0101

CARPI, G., LUIGI, B., ELENA, P., FRANCESCA, C. and RIZZOLI, A. 2009. *Anaplasma phagcytophilum groEL* gene heterogeneity in *Ixodes ricinus* larvae feeding on roe deer in Northeastern Italy. *Vector-borne and Zoonotic Diseases*, (in press). EDEN0105

JURICOVÁ, Z. and HUBÁLEK, Z. 2009. Serologic survey of the wild boar (*Sus scrofa*) for *Borrelia burgdorferi* sensu lato, (submitted). EDEN0108

NETELER, M. Free GIS Software meets zoonotic diseases: From raw data to ecological indicators. EDEN0109

RANDOLPH, S.E., ASOKLIENE, L., AVSIC-ZUPANC, T., BORMANE, A., BURRI, C., GERN, L., GOLOVLJOVA, I., HUBALEK, Z., KNAP, N., KONDRUSIK, M., KUPCA, A., PEJCOCH, M., VASILENKO, V. and ŽYGUTIENE, M. 2008. Variable spikes in tick-borne encephalitis incidence in 2006 independent of variable tick abundance but related to weather. *Parasites & Vectors* (in press). EDEN0112

SIKUTOVA, S., HORNOK, S., HUBALEK, Z., DOLEZALKOVA, I., JURICOVA, Z and RUDOLF, I. 2009. Serological survey of domestic animals for tick-borne encephalitis and Bhanja viruses in northeastern Hungary. *Veterinary Microbiology*. (in press). EDEN0117

Publications of EDEN-TBD (EDEN number not applied for)

SERDT, M., Graduation thesis, University of Ljubljana, Slovenia: Interdepartmental program in Microbiology: European sheep tick *Ixodes ricinus* as a vector of tick-borne bacterium *Anaplasma phagocytophilum* in Slovenia.

COIPAN, E.C., VLADIMIRESCU, A.F., ARSENE, M. and NASTASE, S. 2008. Climate variables influence on the questing activity of Ixodes ricinus ticks in Tulcea County. *Lucrari stiintifice* **51**(10), 267-274. (Medicina veterinara, partea I. Ed. Ion Ionescu de la Brad, Iasi. ISSN 1454-7406).

Meetings where papers involving EDEN-TBD ideas have been presented

RIZZOLI, A. ET AL European Conference of Mathematical and Theoretical Biology, Edinburgh (UK), July.2008.

RIZZOLI, A. ET AL Workshop, Paris, Oct.2008: R_0 and related concepts: methods and illustrations,

RIZZOLI, A. ET AL FAO meeting, Rome, Oct.2008: Climate Change & its Health Impacts on Food/Water Safety and Nutrition.

KAZIMÍROVÁ, M., KOČI, J., DERDÁKOVÁ, M., STANKO, M., TARAGEĽOVÁ, V., SELYEMOVÁ, D., CÍGLEROVÁ, I., LENČÁKOVÁ, D. and †LABUDA, M., VIII České a slovenské parazitologické dny, Sezimovo Ústí, Czech Republic, 19–23.May.2008: Presentation: *Kliešte a kliešťami prenášané patogény na Slovensku sledované v rámci projektu EDEN [Ticks and tick-borne pathogens in Slovakia investigated in the frame of the EDEN project].*

CÍGLEROVÁ, I., TARAGEĽOVÁ, V., DERDÁKOVÁ, M., PEŤKO, B. and KAZIMÍROVÁ, M., Zoonózy – Spoločná ochrana zdravia ľudí a zdravia zvierat, Bratislava, 11-12.Sept.2008: Oral presentation: *Šírenie* *kliešťa obyčajného (Ixodes ricinus) a kliešťami prenášaných patogénov do vyšších nadmorských výšok na území Slovenska [Spreading of Ixodes ricinus and tick-borne pathogens to higher altitudes in Slovakia].*

KAZIMÍROVÁ, M., KOČI, J., DERDÁKOVÁ, M., TARAGEĽOVÁ, V., STANKO, M., SELYEMOVÁ, D., CÍGLEROVÁ, I., MAJLÁTHOVÁ, V., LENČÁKOVÁ, D., VICHOVÁ, B. and †LABUDA, M., Zoonózy – Spoločná ochrana zdravia ľudí a zdravia zvierat, Bratislava, 11-12.Sept.2008: Oral presentation: *Výskum kliešťami prenášaných patogénov na Slovensku v podmienkach globálnych zmien [Study of tick-borne pathogens in Slovakia under global changes].*

KOČI, J., TARAGEĽOVÁ, V., DERDÁKOVÁ, M., SELYEMOVÁ, D., CÍGLEROVÁ, I., LENČÁKOVÁ, D., MAJLÁTHOVÁ, V., VICHOVÁ, B., STANKO, M., KAZIMÍROVÁ, M. and †LABUDA, M. VI International Conference on Ticks and Tick-borne Pathogens (TTP6), Buenos Aires, Argentina, 22-26.Sept.2008: Poster*: Tick seasonal dynamics and prevalence of tick-borne pathogens in Slovakia.*

KOČI, J., DERDÁKOVÁ, M., TARAGEĽOVÁ, V., SELYEMOVÁ, D., CÍGLEROVÁ, I., STANKO, M., MAJLÁTHOVÁ, V., LENČÁKOVÁ, D., VICHOVÁ, B., KAZIMÍROVÁ, M. and †LABUDA, M., Prírodne ohniskové nákazy, 3.-5.Nov.2008, Košice: Oral presentation: *Kliešte a kliešťami prenášané patogény na Slovensku sledované v rámci projektu EDEN. [Ticks and tick-borne pathogens in Slovakia investigated in the frame of the EDEN project].*

VASILENKO, V. and GOLOVLJOVA, I. 13th Baltic-Nordic Conference on Tick-Borne Zoonosis, Kuressaare, Estonia, April.200, Poster: *TBEV and other tick-borne pathogens in Estonia.*

VASILENKO, V. and GOLOVLJOVA, I. Conference on Neuroinfections, Belostok Oct. 2008, Poster: *Unique features of in Baltic TBEV strains within S-TBEV subtype and W-TBEV subtype.*

VASILENKO, V. and GOLOVLJOVA, I. 10th Nordic Viral Zoonoses Network workshop, Seili, Finland, Oct.2008, Poster: *EDEN and VISBY projects in Estonia.*

VASILENKO, V. and GOLOVLJOVA, I. IV Congress Russian Society of Parasitologists ("Parasitology in XXI century – problems, methods, solutions"), Russian Academy of Sciences, Zoological Institute RAS, St. Petersburg, Oct.2008, Poster: *The influence of the different ecological factors on the spatio-temporal changes in the incidence in area of I. ricinus and I. persulcatus.*

VASILENKO, V. and GOLOVLJOVA, I. Conference of Infectious Diseases, Tartu, Estonia, Oct.2008: Report: *Characteristics of zoonotic pathogens in Estonia.*

KNAP, N., DURMIŠI, E., SAKSIDA, A., DUH, D. and AVŠIČ-ŽUPANC, T. VI International Conference on Ticks and Tick-borne Pathogens (TTP6), Buenos Aires, Argentina, 22-26.Sept.2008, Poster: *Influence of climatic factors on dynamics of questing Ixodes ricinus ticks and molecular epidemiology of Tick-borne encephalitis virus in ticks in Slovenia.*

BARANDIKA, J.F., HURTADO, A., GARCÍA-SANMARTÍN, J., JUSTE, R.A., ANDA, P. and GARCÍA-PÉREZ, A.L. 5th International Conference on Rickettsiae and Rickettsial Diseases. Marsella (France), May.2008: Submitted Manuscript: *Prevalence of tick-borne zoonotic bacteria in questing adult ticks from Northern Spain.*

VLADIMIRESCU, A.F. and COIPAN, E.C. 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia, July.2008, Poster: Synchronous seasonal activity of Ixodes ricinus immature stages in Brateiu, Sibiu county, Romania – implications for TBE outbreaks.

COIPAN, E.C, ALEXSE, A., VLADIMIRESCU, A.F. and NICOLESCU, G. 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia, July.2008, Poster: Seropositivity among human subjects for both TBEV and Borrelia burgdorferi s.l. during a TBE outbreak in Sibiu county, Romania.

IONESCU, L. ALEXSE, A., NECSULESCU, M., CEIANU, C., POPESCU, D., BICHERU, S., ORDEANU, V., NICOLESCU, G., VLADIMIRESCU, A.L., HERTZOG, R., POSTOARCA, A. and DUMITRESCU, G. Anniversary Symposium "Medical-Military Scientific Research Centre – 40 years of activity", Bucharest, Romania, Oct.2008, Poster: *The tick-borne encephalitis virus infection research.*

DE MENDONÇA, P.G., KUPCA, A., RACZYNSKI, J., RINDER, M. AND PFISTER, K. Annual Parasitology Meeting of the German Veterinary Society, Celle, Germany, 9-11.July.2008: Paper: *Novel approaches to the epidemiology of anaplasmosis.*

RANDOLPH S.E. International Zoonoses Conference, Glasgow, Scotland, "From Science to Policy", 5-7.Nov.2008, Invited talk: *Potential Effects of Climate and Socio-economic Change on Vector Borne Disease in Europe.*

RANDOLPH S.E. Gordon Conference on Spirochete Biology, California, USA, 21-25.Jan.2008, Invited talk: *Climate change and emerging infections.*

RANDOLPH S.E. German-Austrian Congress for Infectious and Tropical Diseases, Innsbruck, Austria, 27.Feb.-1.March.2008, Invited talk: *Vector-borne diseases and climate change*.

RANDOLPH S.E. 18th European Conference on Clinical Microbiology and Infectious Diseases, Barcelona, Spain, 19–22.April.2008, Keynote Lecture: *Are emerging infections driven by climate change?*

RANDOLPH S.E. ESCMID Conference on Viral Haemorrhagic Fevers, Istanbul, 27-28.June.2008, Invited talk: *Ecology of tick-borne disease and the role of climate*.

RANDOLPH S.E. VI International Conference on Ticks and Tick-borne Pathogens (TTP6), Buenos Aires, Argentina, 22-26.Sept.2008, Keynote Lecture: *To what extent has climate change contributed to the recent epidemiology of tick-borne diseases?*

RANDOLPH S.E. Joint meeting of Belgian and Dutch Societies for Parasitology, Antwerp, Belgium, 6.Oct.2008, invited talk: *Are emerging infections driven by climate change?*

RANDOLPH S.E. Keystone Symposium on Pathogenesis and Control of Emerging Infections and Drug-Resistant Organisms, Bangkok, Thailand, 22-25.Oct.2008, Plenary lecture: *Predicting arrival, establishment and spread of exotic diseases.*

TBD personnel exchanges during reporting period include:

Natasa Knap (IMI) to Dr Lise Gern's laboratory (Neuchâtel).

Dr Philippe de Mendonça (LMU) to Dr Maceij Kondrusik (DIDN)

WP TBD 1 – Landscape, biotopes and habitats

Work package number	TBD1	**Start date or starting event:**	37			
Participant id	ZOOX	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant	1	1	1	1	1	1	1
Participant id	IMI	NEIKER	OEK	PHA	CCDPC	NIRDMI	Total
Person-months / participant	1	1	1	1	1	1	13

Objectives

To complete the acquisition and collation of information on climate and landscape changes in relation to regional and local changes in the incidence of TBDs, specifically tick-borne encephalitis (TBE).

Work performed during previous reporting periods

A database of standardized climatic data from selected sites in each partner country over the period 1970-2004 has been built. Almost all partners have completed the acquisition of daily min and max temperature, rainfall, snow cover. Time-series analyses have been completed and published for the Baltic States. Land cover/land use change in the five selected (CEE) countries have been investigated and indicate a modest increase in the overall extent of forests and woodlands and a very marked abrupt decrease of agricultural activities and productivity in some Eastern European countries after the end of Communism. In a collaboration between EDEN-TBD and HIT-HRRS (partner UCL), a land cover map of NE Latvia has been developed based on Landsat imagery, and validated by field work.

Description of work

Work package number	TBD1	**Start date or starting event:**			37		
Participant id	ZOOX	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant	1	1	1	1	1	1	1
Participant id	IMI	NEIKER	OEK	PHA	CCDPC	NIRDMI	Total
Person-months / participant	1	1	1	1	1	1	13

Objectives

To complete the acquisition and collation of information on climate and landscape changes in relation to regional and local changes in the incidence of TBDs, specifically tick-borne encephalitis (TBE).

Work performed during previous reporting periods

A database of standardized climatic data from selected sites in each partner country over the period 1970-2004 has been built. Almost all partners have completed the acquisition of daily min and max temperature, rainfall, snow cover. Time-series analyses have been completed and published for the Baltic States. Land cover/land use change in the five selected (CEE) countries have been investigated and indicate a modest increase in the overall extent of forests and woodlands and a very marked abrupt decrease of agricultural activities and productivity in some Eastern European countries after the end of Communism. In a collaboration between EDEN-TBD and HIT-HRRS (partner UCL), a land cover map of NE Latvia has been developed based on Landsat imagery, and validated by field work.

Description of work

The climate database will be updated (by all partners) with records for 2005-2008, since most of the archival records were first acquired, to provide data for the period of fieldwork on tick seasonal dynamics.

Achieved results: Meteorological records have been up-dated to the end of 2007 or the first part of 2008 for the following countries: Czechland (IVB), Slovenia (IMI) and Romania (NIRDMI); other partners will send complete record to the end of 2008 early next year. Meteorological records for 1970-2007 for some additional sites were downloaded from the European Climate Assessment web site (http://eca.knmi.nl) for the years 1970-2007 and collated into the existing EDEN-TBD database (ZOOX). (**D TBD 01**).

Further analyses will be carried out to answer specific questions as they arise, with special emphasis on drawing comparisons between changing climatic conditions in Western, Central and Eastern Europe (principally by ZOOX).

Achieved results: Characterization of the precise weather patterns during 2005-2007 in relation to a dramatic atypical spike in TBE incidence in Switzerland, Germany, Slovenia and Czechland, but not other countries, revealed that Jan-March 2006 was unusually cold relative to the past two decades, but July 2006 to May 2007 was exceptionally warm apart from a very cold August (Fig TBD01). The significance of this weather for the observed dynamics of tick populations and TBE epidemiology in eight countries is discussed below (WP5).

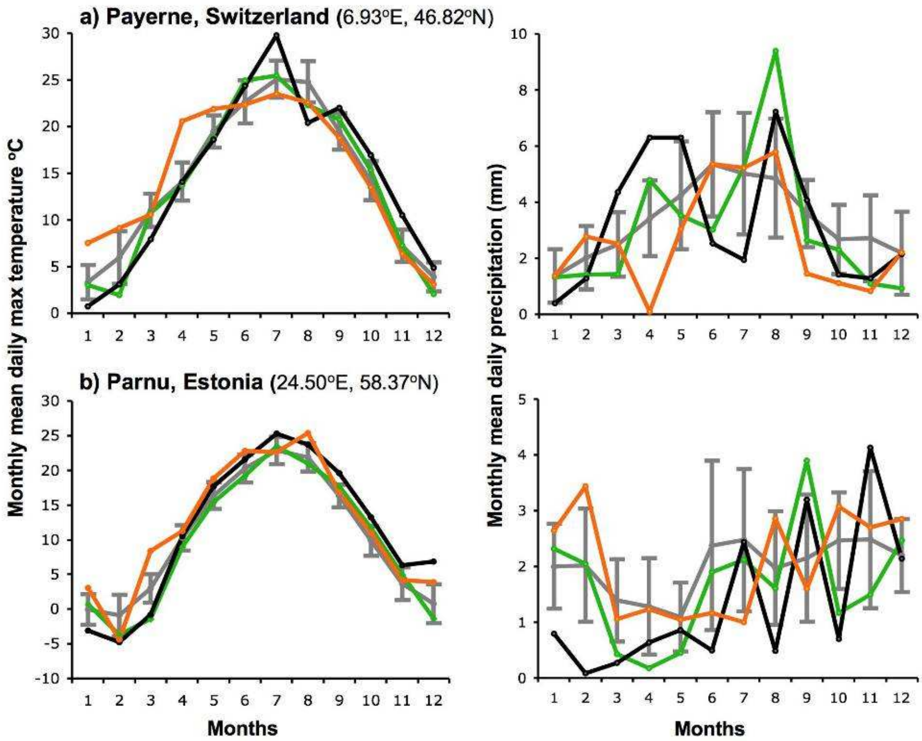

Fig TBD01**:** Monthly means of daily maximum temperature (left column) and daily precipitation (right column) in 1989-07 at two representative locations in Europe. Conditions in 2005 (green), 2006 (black) and 2007 (gold) are shown relative to means ± 1 st dev for the whole period of 1989-07 (grey). Payerne, is close to tick sampling sites within the canton of Bern in Switzerland. Parnu is taken as representative of Estonia across which tick sampling sites were scattered.

Landcover/land use at local scales will be investigated as far as the data permit. Special efforts will be made in those countries where TBE is apparently limited in its range to only part of each country (e.g Poland DIDN, Hungary OEK, Slovakia SAS).

Achieved results: New data on forest coverage in Romania (NIRDMI) have been gathered and will be collated with data on the changing percentages of different land covers in other countries. A full database is being compiled.

Classification of land cover, land use and land tenure with respect to tick habitats and the risk of TBE infection in rural parishes across all of Latvia (based on data from the European CORINE Land Cover project (http://terrestrial.eionet.eu.int/CLC2000)) and in Vidzeme region of NE Latvia (based on Landsat satellite imagery) has proved to be a very demanding task requiring the full dedication of a member of HIT-HRRS (UCL). In addition to metrics derived from maps, census data for populations and land ownership were gathered from the Central Statistical Bureau, the State Forest Service, and the State Land Service. A novel comprehensive landscape analysis was implemented, in which physical structure, socio-economic determinants of landscape use, and human-related "intangible" regulations were all taken into account. Land cover is related to disease ecology, land use to human exposure, and land tenure/access to the institutional control on the potential overlap between the two previous factors. These elements, for the case of rural Latvia, are shown in Fig TBD02. The variables investigated in Latvia, and those which proved to be significant in multivariate models, are shown in Tables TBD01 & TBD02. This work has now been written up for publication (UCL, PHA, ZOOX). The level of expertise and extra fieldwork needed to achieve this sort of in-depth explanation of TBE risk at the parish level is beyond the scope and resources available to other EDEN-TBD partners. The completed analysis for Latvia should, however, act as a model for any future work within any European country for which this is seen as a priority to justify the necessary funds. (**D TBD 02**).

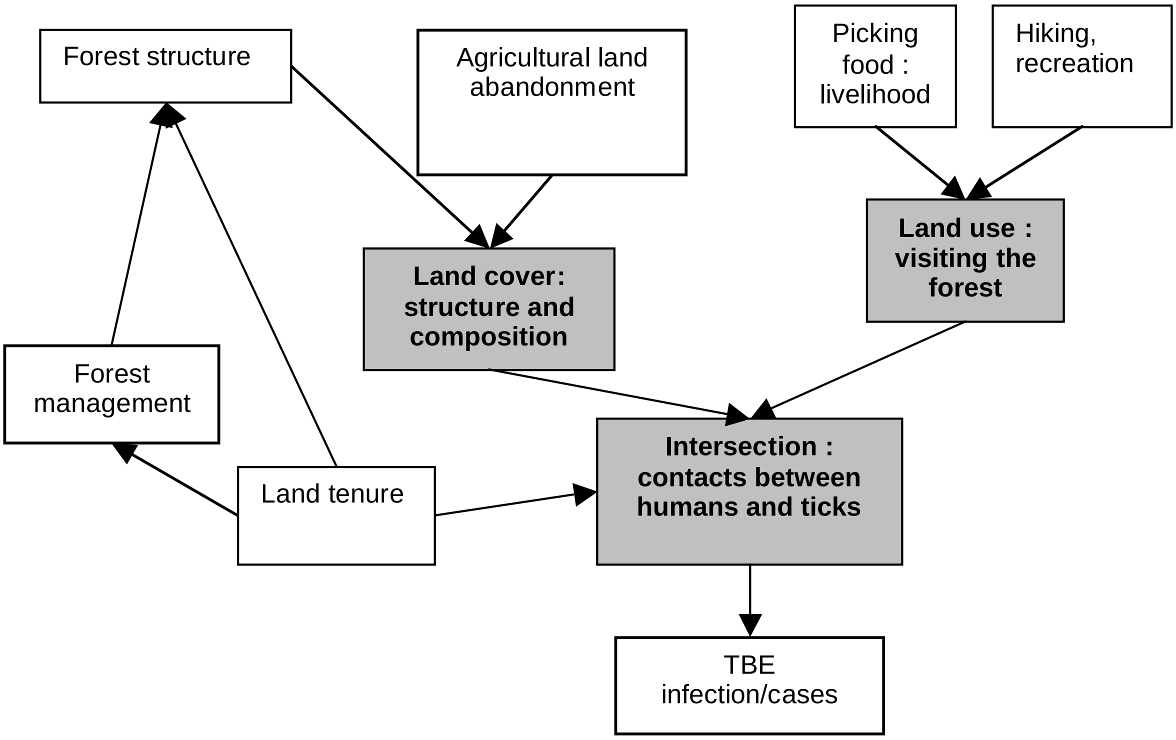

Fig TBD02: Hypothesised relationship between TBE and land cover, land use and their intersection.

Table TBD01: Variables tested as hypothesised risk factors for TBE in Latvia.

Hypothesis group	Variables selected (with data source)	Hypothesized sign of relationship with the presence of TBE cases
Land cover	% parish covered with forest §	+
	% forest regeneration that is natural regeneration *	+
	Mean area of forest patches §	+
	Mean Shape Index of forest patches §	+
	% parish covered with clear-cut§	+
	Mixed and transitional vegetation around forests §	+
	% parish covered with mixed disturbed vegetation §	+
	% parish covered with transitional forest §	+
	% agricultural land under fallow #	+
	% agricultural land that is arable #	–
Land use	Mean area per farm#	–
	% population with an economic activity°	Hikers + /Pickers –
	% population receiving social assistance°	Hikers – /Pickers +
	% population receiving a pension°	Hikers – /Pickers +
	% population with higher education°	Hikers + /Pickers –
	% population with <4 years of formal education°	Hikers – /Pickers +
Land tenure	% forest area managed by the state/other managers*	+/ –
	% parish area managed by the state/other managers*	+/ –

*§ land cover map; * State Forest Service; # agricultural census; ° population census*

Table TBD02: Summarised results of the four multivariate models to explain TBE presence in rural parishes at the national level and for Vidzeme region in Latvia.

Non-spatial multivariate model Autologistic multivariate model

National level	% forest coverage	+	% forest coverage	+
	% forest felled	+		
	% agricultural land around forest	-		
	% with a pension	-	% with economic activity	-
	% with higher education	+	% with higher education	+
	% non-state forest	-	% state forest	+
			neighbouring parishes with TBE	+
	Pseudo-r²=0.07		Pseudo-r²=0.14	
Vidzeme region	Ratio of clear-cut forest	-	Ratio of clear-cut forest	-
	% arable land	-	% arable land	-
	% <4 years of education	+	% <4 years of education	+
			% non-state forest	-
			neighbouring parishes with TBE	+
	Pseudo-r²=0.12		Pseudo-r²=0.30	

Plus and minus signs indicate the sign of the relationship.

Deliverables

D TBD01 – Update of spatial database of standardized climatic data for each TBD partner country at multinational levels (M18-24-36-48). Level of achievement, 80%.

D TBD02 – Spatial database of land cover/land use change and initial analysis maps for selected countries (e.g. the Baltic States) at the national, sub-national and local scale (M18-24-36-48). Level of achievement, 100% for Latvia.

Milestones and expected result

M TBD18 – The spatial database on climatic data and land cover/land use change are periodically updated. (M36-48) Level of achievement, 100%.

WP TBD 2 – Vector bionomics and competence

Work package number	TBD2	Start date or starting event:				37		
Participant id	IVB	NIRDMI	CEA-FEM	DIDN	LMU	SAS	TAI	CNM
Person-months/ participant	23	23	13	23	33	23	23	7.5
Participant id	IMI	NEIKER	OEK	PHA	CCDPC	VRMI		Total
Person-months/participant	23	15	0	32	28	29		295.5

Objectives

The general objective of this WP is to link tick population changes and dynamics with regional and local changes in the incidence of TBDs in the cluster of countries with resident EDEN-TBD teams. During the period of months 25-42 the specific objectives are:

- To complete the development and dissemination to each TBD partner institute state-of-the-art molecular tools and standardized protocols for (i) diagnosis of multiple infections in ticks and (ii) identification of tick blood meals.
- To analyze at the national and sub-national scale historical tick population changes in relation to regional and local changes in the incidence of TBDs.
- To undertake fieldwork to collect data on different patterns of tick seasonal population dynamics (processes) associated with different epidemiological patterns in each partner country.
- To analyse samples of field-collected ticks from each site for their infection prevalence and blood meal identity.

Work performed during previous reporting periods

The German team (LMU), together with the Slovenian (IMI) and Swiss teams, has developed molecular tools and standard protocols for the detection of pathogens in ticks, and is continuing to develop tools for the identification of tick blood meals. In addition to the 3 training workshops on Real Time PCR during period 1, exchanges between Munich and various teams have ensured adequate further training for those who need it. Most teams have started to process ticks, as far as limited funds permit, and some preliminary data on infection prevalence have been achieved.

Each partner has searched for available data on changes in tick populations from 1970 to the present. This search will go on, as new sources are continually coming to light. Available data are very variable in terms of quality and quantity. Eighty one field sites have being selected for monthly tick sampling over periods 2, 3 and 4. The sampling protocols have been defined and implemented. The planned numbers of nymphal and adult ticks have been collected from each site and stored (frozen) for analysis of their infection status and blood meal identity once the molecular diagnostic protocols are finalized. Ongoing analyses have revealed major increases in tick abundance in some areas, and highly variable patterns of tick seasonal dynamics from year to year and place to place, as expected.

Description of work

Monthly tick sampling will continue throughout the period M37-M48 to complete the full three years of observations (NIRDMI, IVB, CEA-FEM, DIDN, LMU, SAS, TAI, IMI, NEIKER, PHA, CCDPC, VRMI, Neuchâtel).

Achieved results: All partners have now achieved the third full year of tick sampling to assess the variable patterns of seasonal population dynamics at 4-8 sites per country, and to investigate the infection prevalence with four different types of pathogens. The tick population data have been sent to Oxford to be added to the database already on the EDEN website (ZOOX). Analysis of data for years one and two shows that the unusually warm spring of 2007 caused an earlier onset of tick activity, with nymphs appearing once the decadal mean daily maximum temperature had crossed the 7^{o}C threshold (Fig TBD03). The total annual abundance of ticks, however, was the same for 2007 and 2006 in 41% of sites, lower in 32% and greater in 27%. The data for 2005 (Slovenia only), 2006 and 2007 have already proved invaluable in an investigation of the unusual epidemiology of TBE over these years (see WP5 below).

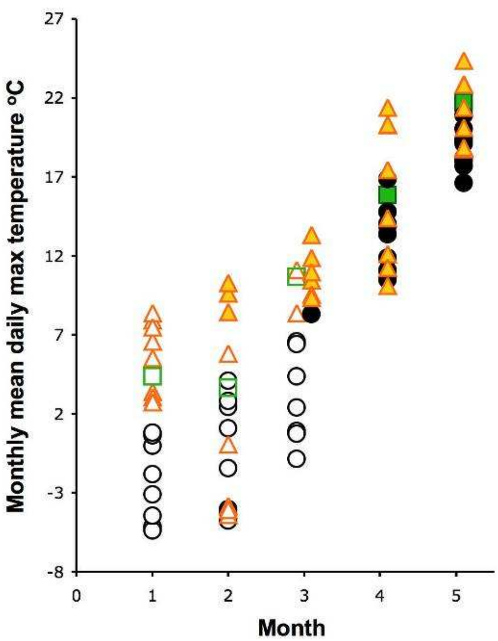

Fig TBD03: Month of onset of activity by *Ixodes ricinus* nymphs in relation to monthly mean daily maximum temperature. Averages for 4-7 tick-monitoring sites in each of Switzerland, Germany, Slovenia, Czechland, Poland, Lithuania, Estonia and Latvia. Temperature recorded at locations near to, or within the geographical limits of, tick sampling sites. Inactive ticks (open symbols) or active ticks (closed symbols) for 2005 (green square), 2006 (black circle) and 2007 (gold triangle).

Molecular diagnosis of infection in samples of ticks collected at each site in 2007 (or 2006) will be completed (same partners + CNM).

Achieved results: Most partners have now deployed the protocols for the detection of the following pathogens: TBE virus, *Anaplasma, Borrelia burgdorferi, B. afzelii, B. garinii, B. valaisiana*, *B. lusitaniae, Babesia microti and Babesia divergens* (**D TBD 16**). IVB have started RNA and DNA extraction and NIRDMI now have a newly completed molecular diagnostic facility; both are now ready to start diagnosis in early 2009. Other partners have now processed many thousands of ticks to variable degrees of completion. On-going quality control by LMU is currently dealing with samples from Slovakia, Lithuania and Poland.

- TBEV: as expected, the prevalence is very low (0-1.3%), especially in nymphal ticks; the inevitable compromise between large enough sample sizes and prohibitive costs was always recognized. Sequencing and subsequent BLAST analysis confirmed that samples from Slovakia (SAS) possessed the highest identity with HYPR strain of TBEV; in northern Italy (CEA-FEM), all TBEV positive samples isolated belong to the western European TBEV subtype.

- *Anaplasma phagocytophilum*: the infection prevalence is higher, with significantly higher prevalence in adult ticks (up to 7.1%) than nymphs (up to 2.1%), as expected. Genetic heterogeneity amongst *A. phagocytophilum* isolates from larval ticks in northern Italy (publication EDEN0105).

- *Borrelia burgdorferi* s.l.: infection prevalences fall within the expected range, usually close to 10% overall. The data from Slovakia (SAS) provide a good example of the detailed results available to date. Total *B. burgdorferi* s.l. infection prevalence within western Slovakia reached 25.9% and included the following most prevalent genospecies: *B. garinii* (31%), *B. afzelii* + *B. burgdorferi* sensu stricto co-infection (26%), *B. valaisiana* (14%) and *B. garinii* + *B. lusitaniae* co-infection (7%). *B. burgdorferi* s.l. predominated at site Medné (38%).

- *Babesia* sp: infection prevalence varied from 0.53% in 2005 to 3.17% in 2006 in Slovenia (IMI); it reached 4.5% overall in Hungary (VRMI), and 2.7% in Slovakia (SAS). More detailed results for each site within countries are available. In Slovakia, for example, *Babesia* (and *Anaplasma*) was most prevalent at site Malacky. RLB genotyping revealed that 16 (48%) of the *Babesia*-positive samples hybridised with neither *B. microti* nor *B. divergens* RLB probes, but they hybridised only with the universal *Babesia* spp. probe. 14 samples (42%) were genotyped as *B. microti*, 2 samples (6%) as *B. divergens* and one sample (3%) was co-infected by both *Babesia* genospecies.

The following results from Italy (FEM) give an indication of the variation in infection prevalence between sites (Table TBD03).

Table TBD03 - Mean prevalence of TBEV recorded in pools of nymphs and single adults of I. ricinus in Northern Italy, collected during 2006.

ID_Site	Site	Region	Province	Pools of nymphs / TBEV +ve	Prevalence %	No. Adults/ TBEV +ve	Prevalence %
IT1	Monticolo	Trentino	Bolzano	21/0	0.0	32/0	0.0
IT2	Lamar	Trentino	Trento	41/0	0.0	42/2	4.7
IT3	Filari	Trentino	Trento	58/0	0.0	6/0	0.0
IT4	Candaten	Veneto	Belluno	56/0	0.0	66/0	3.0
IT5	Losego	Veneto	Belluno	61/0	0.0	33/0	0.0
Total				237/0	0.0	179/3	1.68

Mean prevalence of A. phagocytophilum recorded in pools of nymphs and single adults of I. ricinus in Northern Italy, collected during 2006.

ID_Site	Site	Region	Province	Pools of nymphs/ TBEV +ve	Prevalence %	No. Adults/ TBEV +ve	Prevalence %
IT1	Monticolo	Trentino	Bolzano	21/2	1.98	32/2	6.25
IT2	Lamar	Trentino	Trento	41/0	0.0	42/3	7.14
IT3	Filari	Trentino	Trento	58/2	0.70	6/0	0.0
IT4	Candaten	Veneto	Belluno	56/0	0.0	66/0	0.0
IT5	Losego	Veneto	Belluno	61/6	2.05	33/0	0.0
Total				237/10	0.86	179/5	2.80

The successful method of blood meal analysis recently developed by the Swiss associate team (Neuchâtel) will be further developed and applied to ticks collected at selected sites within the EDEN-TBD project (depending on resources: LMU, NEIKER, IVB, SAS, NIRDMI).

Achieved results: These analyses are ongoing in Germany (LMU) and Switzerland (Neuchâtel), but limited funds have prevented similar work in Spain (NEIKER). Other partners listed above still express an interest in pursuing these analyses.

Deliverables D TBD16 – Final measures of the geographically variable prevalence of infection with tick-borne pathogens in ticks (M54). Level of achievement, 50%. 2 new publications (EDEN publication numbers EDEN0099 and EDEN0105). D TBD17 – Information on tick blood meal identity in relation to infection prevalence (M54). Level of achievement, 10%.
Milestones and expected result M TBD14 - The first molecular diagnostic data to indicate the sensitivity and specificity of the methods (M36). Level of achievement, 100%.

WP TBD 3 – Public health and human activities

Work package number	TBD3	Start date or starting event:		37			
Participant id	NIRDMI	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant	1	1	1	1	1	1	1
Participant id	IMI	CNM	OEK	PHA	CCDPC	ZOOX	Total
Person-months / participant	1	1	1	1	1	1	13
Objectives To complete the acquisition and collation of information on public health activities and sociological factors relevant to human exposure to infected ticks, in relation to regional and local changes in the incidence of TBDs, specifically tick-borne encephalitis (TBE).							

Work performed during previous reporting periods

Records of TBDs (principally TBE) at local and regional scales over the period 1970-present were extracted from national Public Health agencies by most partners. Detailed data on such factors as the age and sex, and sociological markers of potential changes in human behaviour associated with agricultural practices, poverty and wealth, have been proved relevant to an increased risk of exposure to ticks. In addition, almost all partners have also recorded relevant public health information, including an analysis of the impact of improved methods of diagnosis on official national records or vaccination coverage. From the growing bank of data that has been accumulated, an increasingly clear picture has emerged of the changes in public health activities (principally vaccination) and sociological factors related to contact between humans and infected ticks in forests over the past 35 years.

Description of work

Data on incidence of tick-borne disease incidence in each county within the EDEN-TBD area will be up-dated (ZOOX) as annual records become available, to allow the epidemiology to be related to contemporary tick dynamics from field samples (WP2) as well as long-term trends in environmental and sociolo-economic conditions (M37-54) (all partners). As the analysis at local, national and international levels proceeds, additional data will be sought to answer specific questions as they arise (all partners).

Achieved results: annual data on the incidence of TBDs have been up-dated, although not yet complete for 2008. Further details concerning the dynamic epidemiology of TBE have been gathered, particularly with reference to seasonal patterns of incidence. These were used to quantify the variable degrees of mis-match in the timing of the seasonality of ticks and human TBE cases, which might indicate the effects of human behaviour on exposure to infected ticks in forests (see WP5 below). These data were used to investigate the unusual spike in TBE incidence in 2006 in some, but not all countries (Table TBD04).

Table TBD04 - Annual TBE cases 2005-07, compared with means over the previous decade

	Annual TBE cases				
	1995-04 mean	± 1 st dev	2005	2006	2007
Switzerland	92	± 29	208	245	111
Germany	193	± 72	432	546	238
Slovenia	231	± 81	297	445	196
Czechland	574	± 115	643	1029	546
Poland	214	± 71	174	316	233
Lithuania	416	± 194	242	462	234
Slovakia	75	± 15	50	91	46
Italy	14	± 9	19	30	17
France	3	± 3	0	6	0
Sweden	94	± 39	130	163	190
Norway	2	± 1	0	5	12
Estonia	233	± 98	164	171	140
Latvia	593	± 388	142	170	171
Finland	24	± 11	17	18	20
Hungary	117	± 71	52	56	62

Valuable data on numbers of visitors to a major national park in Slovenia over the past four years were found, and indicated greater recreational activity in 2006, presumably in response to the unusually clement weather.

In addition, data on sex and age distributions of infected people have been strengthened, with a view to detecting any annual changes in relation to extrinsic factors.

Additional data for Romania (NIRDMI) on poverty and wealth indicators, and agricultural practices have been gathered and will be added to the database.

All new data are continually being added to the already extensive database on sociological indices relevant to tick-borne disease systems (**D TBD 08**).

It was confirmed that the abrupt upsurge in tick-borne encephalitis (TBE) incidence in the newly independent Baltic States was not due merely to changes within the public health system. Relevant practices of surveillance, registration, diagnosis, awareness and immunisation were documented from archived data and interviews with experienced medical practitioners. There were changes that could have had neutral, negative or positive effects on recorded TBE incidence, but the variable timing in these changes at both national and regional scales is not consistent with their having been responsible for the epidemiological patterns in the early 1990s (publication EDEN0101).

Deliverables

D TBD08 – Standardized database of national records of historical trends in sociological indices of agricultural practices, poverty, wealth, and food harvest and leisure activities in (potentially) tick-infested forests, 1970-present, in each TBD country (M26). Level of achievement: detailed data collection increasingly complete, but there is no definitive end-point to the discovery of archived data; compilation into a standardized database continuing. 85% complete.

D TBD23 – Analysis of public health activities in relation to TBE upsurge in the Baltic States (M42). Level of achievement, 100%. 1 new publication (EDEN publication number 0101).

Milestones and expected result

Spatial patterns in sociological factors and land cover/land use (WP1) in relation to epidemiological data will direct the further selection of particular indices of sociological and behavioural change that may have increased human contact with infected ticks (M26). Achieved.

WP TBD 4 – Animal reservoirs

Work package number	TBD4	**Start date or starting event:**			37		
Participant id	NIRDMI	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant	1	1	9	1	1	9	1
Participant id	IMI	NEIKER	OEK	PHA	CCDPC	ZOOX	Total
Person-months / participant	1	1	1	1	1	1	29

Objectives

The general objective of this WP is to describe the impact of environmental changes or human intervention on host availability and the abundance and/or seasonal dynamics of local tick populations. During months 25-42 the specific objective will be:

- To map regional and local changes in the distribution and abundance of wildlife and livestock vertebrate hosts for ticks.
- To continue to collect field data to establish the quantitative relationship between variable ungulate host availability on tick infestation patterns on rodents and infection prevalence in ticks and rodents.

Work performed during previous reporting periods

A large amount of valuable data on large animal hosts for ticks, both domestic livestock (cattle, sheep, goats) and wildlife species (especially deer and wild boar, but also mouflon and wolves), was acquired and collated. These data have been compiled into standardized databases prior to presenting the information in mapped formats. The importance of the different types of livestock for maintaining tick populations varies from country to country depending on husbandry practices, such as the extent of outdoor grazing and seasonal patterns of grazing at different altitude. In addition, protocols and grids for rodent trapping were established at 3 sites in Italy and at 2 sites in Slovakia. Seasonal variations in the rodent populations and infestation levels of *I. ricinus* larvae and nymphs have been monitored over 2 years, and ticks were collected from these rodents. Diagnosis of infection prevalence with tick-borne pathogens at each site has started. These intensive field studies complement the GIS spatial analyses carried out in Italy, helping to inform us about the possible consequences of changing deer abundance on the transmission potential on various tick-borne pathogens.

Description of work

Rodent sampling will continue in Slovakia (SAS), and infection diagnosis will be completed.

Achieved results: Rodent sera collected in Italy (CEA-FEM) during 2006 were analysed by ELISA (analysis carried out in UK, by Kalon Biological) (Table TBD05). Sera from ca. 1,000 rodents collected in 2007 at the long-term site of Cavedine, northern Italy are currently being analysed in collaboration with the EDEN-ROBO team. The results will be compared with those from Slovakia for 2007.

Table TBD05: Results of sero-survey by ELISA for TBE infection in rodents trapped in northern Italy. All the positives samples came from *A. flavicollis*.

Year	Province	Type of sampling	Site	No. samples	No. tested	TBE positive	% prevalence (± s.e.)
2006	BL	Extensive	Candaten	51	51	1	1.94 (1.94)
2006	SO	Extensive	Grosotto	6	6	0	0
2006	SO	Extensive	Mazzo	47	47	0	0
2006	TN	Extensive	Laghi Lamar	40	40	0	0
2006	TN	Intensive	Cavedine	323	323	9	2.79 (0.92)
2007	TN	Intensive	Cavedine	1027	-	-	-
			Total	**1494**	**467**	**10**	**2.14 (0.67)**

In Slovakia (SAS), small mammals were live-trapped in 2008 at two sites (Rozhanovce and Topoľčianky) (**D TBD 18**). Over 8 trap-nights in April, May, September and October in Topoľčianky and 3 trap-nights in April, May, and October in Rozhanovce, 62 small mammals were captured, 50 in Rozhanovce, but only 12 in Topoľčianky. These comprised 5 species of rodents (*Apodemus agrarius*, *A. flavicollis*, *Clethrionomys glareolus*, *Micromys minutus, Microtus arvalis*) and one species of shrew (*Sorex araneus*). Of 140 ticks collected from these small mammals, all were *I. ricinus* and almost all larvae (only 2 nymphs) Blood samples and skin biopsies taken from almost all animals are being screened for infection with tick-borne pathogens in collaboration with the EDEN-ROBO team. Detailed data on individual rodents for all three years will allow analysis of infection status in relation to host demographic factors and tick infestations.

In addition, the Czech team (IVB) carried out serological surveys in wild and domestic animals. Indirect hemagglutination assay (IHA) for *Borrelia burgdorferi* sensu lato in wild boar (*Sus scrofa*) in Czechland detected antibodies in sera from all 10 regions, with an overall seroprevalence of 12.8% (titres from 1:80 to 1:640). Seroprevalence between administrative regions varied as follows: Moravskoslezský (25.0%), Pardubický (25.0%) and Královehradecký (24.1%), followed by the regions Plzeñský (16.7%), Olomoucký (13.3%), Jihomoravský (12.8%), Vyškovský (11.1%), Jihoceský (11.1%), Zlínský (10.3%) and Liberecký (8.9%) (Fig TBD04) (taken from publication EDEN0108). Seroprevalence increased in March and April to a peak in May. The results suggest frequent exposure of wild boar to ixodid ticks infected with *Bb*, predominantly in rural and forested regions. The precise role of these animals in the ecology of in Lyme borreliosis (LB) is still unknown, but wild boar serology may provide another means of surveillance of endemic areas of LB.

The Czech team (IVB) also examined blood sera collected in 2005 from 260 cattle, 100 Merino sheep and 40 horses in NE Hungary for antibodies to TBE viruses, using ELISA for screening and plaque-reduction neutralization as a confirmatory test. 26.5% of cattle, 7% of sheep and zero horses were sero-positive (publication EDEN0117). Older cattle (>3 yrs) had a significantly higher seroprevalence. The results confirm the presence of natural foci of TBE in some parts of NE Hungary, specifically Nograd and Herves counties, but only in the extreme west of Borsod-Abauj-Zemplen county (despite c.5 human cases of TBE annually in this latter county).

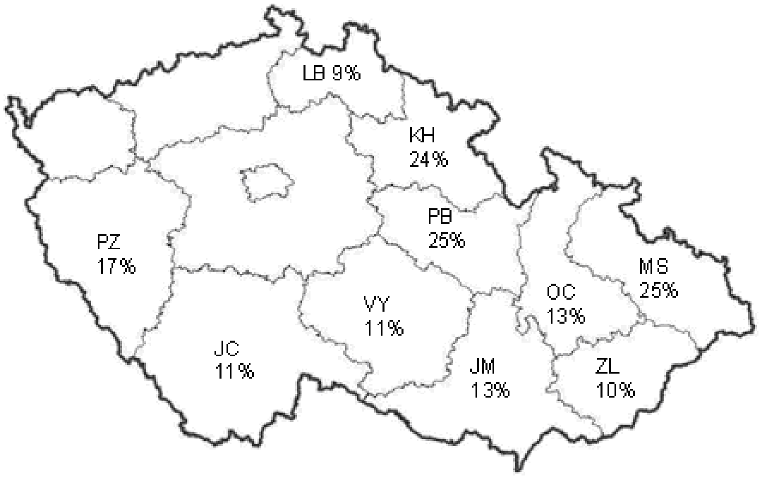

Fig TBD04: Prevalence (%) of *Borrelia burgdorferi* s.l. antibodies in wild boar for in different regions of the Czech Republic.

In Latvia, rodent trapping was carried out in April and October 2008 to test whether rodents had colonized abandoned agricultural land now covered to varying degrees with perennial weeds, shrubs and young trees. Rodent bodies were cryo-preserved for subsequent examination for ticks, TBE virus and rodent-borne viruses infection in Dr Vapalahti's laboratory in Finland. The results will be related to the identified significance of abandoned agricultural land as a predictor of TBE presence (see WP1).

Indices of deer abundance at each tick sampling site will be acquired from local hunters associations, etc by each team (NIRDMI, IVB, CEA-FEM, DIDN, LMU, SAS, TAI, IMI, NEIKER, PHA, CCDPC, VRMI, Neuchâtel).

Achieved results: New data on wildlife abundance in different parts of Romania (NIRDMI) for 2006 and 2007, and on livestock abundance since 1990, have been sent to Oxford for inclusion in the database, and similar data for the period 2004-08 for Spain (NEIKER) have been requested from conservation groups, hunters, farming organisations, etc. (**D TBD 11 and D TBD 12**).

Deliverables D TBD11 – Maps of changing patterns of local and regional deer abundance, 1970-present (M42). Database complete as far as available data allow, and now mapped by HIT-DMT. Level of achievement, 99%. D TBD12 – Maps of changing patterns of local and regional livestock abundance, 1970-present (M42). Database complete as far as available data allow, and now mapped by HIT-DMT. Level of achievement, 99%. D TBD18 – Third annual pattern of rodent and tick population dynamics, and seasonal prevalence of infection (M48). Completed, apart from serology on rodents from Slovakia. Level of achievement, 80%. D TBD 19 – Database in collaboration with Robo and HRRS on changed rodent populations and viral infection prevalence in relation to habitat changes (M36). Fieldwork completed; serology on rodents under way. Level of achievement, 70%. D TBD22 - Indices of deer (and wild boar) abundance at each site where ticks are sampled (M52). No data yet reported. D TBD 24 – Sero-surveys of wildlife as indicators of the presence of zoonotic cycles of TBDs (M48). First results available. 2 new publications (EDEN publication numbers 0108, 0117).
Milestones and expected result M TBD 16 - First results of serological evidence of infection in rodents with TBE virus (M30). Level of

achievement, 50%. M TBD 21 – Identification of rodent trapping sites in Latvia (M42). Fully achieved.

WP TBD 5 – Data management and cross disciplinary modelling

Work package number	TBD5	Start date or starting event:		37		
Participant id	ZOOX					Total
Person-months / participant	10					10

Objectives The general objective of this WP is to use data generated by WP1-2-3-4 to develop epidemiological models of TBD risk to predict changes in TBD systems driven by landscape and climate changes as well as socio-economical changes. During months 13-30 the specific objectives are: • To develop GIS databases of all information acquired from WP1-4. • To develop a model of the predictor variables of the observed epidemiological changes by seeking correlations between patterns of disease and patterns of environmental and sociological factors.

Over the first periods, a huge array of data sets was acquired by each partner and sent to Oxford for collation by partner ZOOX. The first parts of the geo-referenced multi-national databases have been posted on the EDEN web site. Records of the annual incidence of TBE in each administration district in each partner country from 1970-2005 (WP3) were compiled into a common database and presented in both digital and mapped form. Climatic data have also been imported. Databases for information on other factors (land cover [WP1], vectors [WP2], sociological changes [WP3] and hosts [WP4]) are nearing completion. The abundance of the biological and sociological data, together with their non-uniformity, make the compilation of a single database much more complex and time demanding than was first imagined. The timing of statistically significant increases in the incidence of TBE varied between and within countries. No one factor shows consistent correlations with the spatio-temporal patterns of epidemiological change, as most factors showed considerable consistency within any one country. A working hypothesis was developed that emphasizes the synergy between independent, but indirectly linked, socio-economic and human-induced environmental changes in determining the spatio-temporal heterogeneity in TBE upsurges. As more data on environmental and socio-economic variables have been acquired and collated into databases, mostly at national levels but sometimes at county level, the increasing body of evidence has substantiated, and in no way yet falsified, this analytical framework. Explanations for the upsurge of tick-borne diseases in CEE countries must now recognize not only an increase in risk though more infected ticks, but also an increase in human exposure to that risk through changing behaviour.

Description of work

The same analytical framework will be used to try to relate differences in TBE epidemiology to changing environmental and socio-economic conditions within CEE countries, and also in countries in western Europe (ZOOX with input from all partners). Even though the latter have clearly not suffered the same abrupt political transition as in CEE countries, nevertheless more gradual changes in life style relevant to the risk of infection may be identified, quantified and their impact explored. The environmental effects of the collapse of agriculture in some parts of the Baltic States likely to enhance the transmission potential of both TBE virus and Rodent-borne viruses will be explored. In particular, the natural vegetational succession observed in very many abandoned fields may increase the habitat for rodents. This will be investigated by fieldwork in Latvia (ZOOX, PHA, METLA).

Achieved results: In Italy (CEA-FEM), the following GIS data analysis and modelling has been undertaken :

GIS data preparation:

- Trentino GIS Database: A completely new provincial GIS database was created based on the new official high-resolution data. This includes a provincial digital elevation model / DSM at 1m / 2m resolution, a complete orthophoto coverage and various vector layers. Metadata were corrected and projections unified to UTM32N/WGS84. As appropriate, selected maps were reprojected to Longitude-Latitude graticule. For easy data access, tile indices and image pyramids were generated.

- Gazetteer data: new placename databases were generated for Italy and Slovakia which assist in geocoding additional data (data source: geonames.org).
- Satellite data: MODIS Land Surface Temperature (LST) maps were downloaded again from the NASA WIST since their production level V005 reprocessing was completed which includes major data improvements. The MODIS post-processing chain at FEM-CEA was further improved to achieve a high degree of automation. All maps are now reprojected to UTM32N/WGS84 to avoid complex geodetic datum issues with the older Italian Gauss-Boaga system. About 10500 MODIS LST were processed for Northern Italy, i.e. all available maps from Terra (5 mar 2000 - 15 sept 2008) and Aqua satellites (8.July.2002 – 15.Sep.2008) were prepared. Hence, from 8.July.2002 four observations per day are available.
- Climatic data time series from stations already selected were completed and prepared for submission to Oxford.
- A new data set of spatialized daily precipitation data from the GCPC NASA project was converted from the original proprietary to common GIS formats: data are available from 10/1996 to 4/2008, at a spatial resolution of 1^{o}. This data set is appropriate for analysis of precipitation patterns at sub-national to continental scales.

GIS analysis:

- The algorithm to completely reconstruct MODIS Land Surface Temperature (LST) that was initially developed in 2007, was further revised and improved. Given the complex terrain in the alpine regions of Northern Italy, the algorithm now takes into account the temperature gradient to reconstruct cloud contaminated pixels. In case of an insufficient number of pixels in a map, a monthly gradient is applied instead. An additional correction is applied for the exposure of slopes. In the case of incomplete LST maps, the original map with holes and the temperature gradient map are combined and a random sampling is applied to extract a well-distributed number of points. Eventually, these points are re-interpolated with volumetric splines and elevation as auxiliary variable, leading to a complete LST map. Due to the high computational demand, these calculations are performed on a cluster. A significant acceleration of the 3D splines interpolation (less than 40% of CPU time) is currently under review. About 10500 MODIS LST were processed for Northern Italy as well as 3000 maps for the Slovakian study sites (years 2005 and 2006).

EDEN-TBD has been able to capitalize on the exceptional gift of an extraordinary epidemiological event, matched by unusual weather, coinciding with the course of the fieldwork (publication EDEN0112). The incidence of tick-borne encephalitis showed a dramatic spike in several countries in Europe in 2006, a year that was unusually cold in winter but unusually warm and dry in summer and autumn. The possible causes of the sudden increase in disease were tested: more abundant infected ticks and/or increased exposure due to human behaviour, both in response to the weather. The field data on tick abundance for 2005-2007, collected monthly from a total of 41 sites in 8 countries (Switzerland, Germany, Slovenia, Czechland, Poland, Lithuania, Estonia and Latvia) were analysed in relation to total annual and seasonal TBE incidence and temperature and rainfall conditions. The weather in 2006-2007 was exceptional compared with the previous two decades (Fig TBD01, WP1), but neither the very cold start to 2006, nor the very hot period from summer 2006 to late spring 2007 had any consistent impact on tick abundance (Fig TBD05). Furthermore, the TBE spike in 2006 was not related to changes in tick abundance. The degree of TBE spike varied between countries, despite similar weather patterns. The degree to which seasonal variation in TBE incidence matched seasonal tick activity also varied between countries, with disproportionately greater increases in TBE incidence in the autumn, after tick questing activity had declined, in countries with greater cultural traditions of mushroom harvest (Czechland, Poland and the Baltic States). The data suggest that the TBE spike was not due to weather-induced variation in tick population dynamics.

An alternative explanation, supported by qualitative reports and some data, involves human behavioural responses to weather favourable for outdoor recreational activities, including hiking and wild mushroom and berry harvest, differentially influenced by national cultural practices and economic constraints. Sportive recreational activities are especially well developed in Switzerland, Germany and Slovenia; there were indeed more visitors than average to national parks in Slovenia during the unusually warm weather in July, September and October 2006 (see WP3). Mushroom gathering is economically particularly important in NE Poland and Lithuania, from where large quantities of wild edible fungi are exported to Western Europe; activities upon which people depend for their livelihood are likely to vary less by drawing in opportunistic recreational foragers in good years, although TBE cases were disproportionately high in the autumn in 2006 (after good growing conditions in a very wet August). Indeed, in years when the crop is poor, additional efforts may be needed to secure a good harvest. This may explain why the TBE spike in 2006 was no higher than that in some other years in NE Poland and Lithuania, when the excess cases also occurred in the autumn. During 2000-07, only 8% of annual TBE cases in NE Poland and 13% in Lithuania were recorded before July, and only 38-48% before September, suggesting very little human activity in forests before the mushroom season.

Hungary is one of only two countries in Europe (the other being Croatia) to have experienced a sharp decrease in TBE incidence from one year to the next (1996-97). A common view is that this was due to changes in the public health service organization, making TBE diagnosis privately funded and therefore less likely to be sought. To test this, data on numbers of cases of TBE in each village in Hungary from 1970 to 2004 are now partially analysed (ZOOX) to identify epidemiological patterns related to population size, population change, geographical location, etc, all of which could indicate that the likelihood of TBE diagnosis varies with socio-economic development and therefore poverty. A visit to Hungary and Slovenia by the SP leader in July/August 2008 offered the opportunity to witness first hand some of these factors, and the marked contrast in the juxtaposition of human habitation and tick habitats in these two neighbouring countries, that might account for the very different levels of TBE incidence.

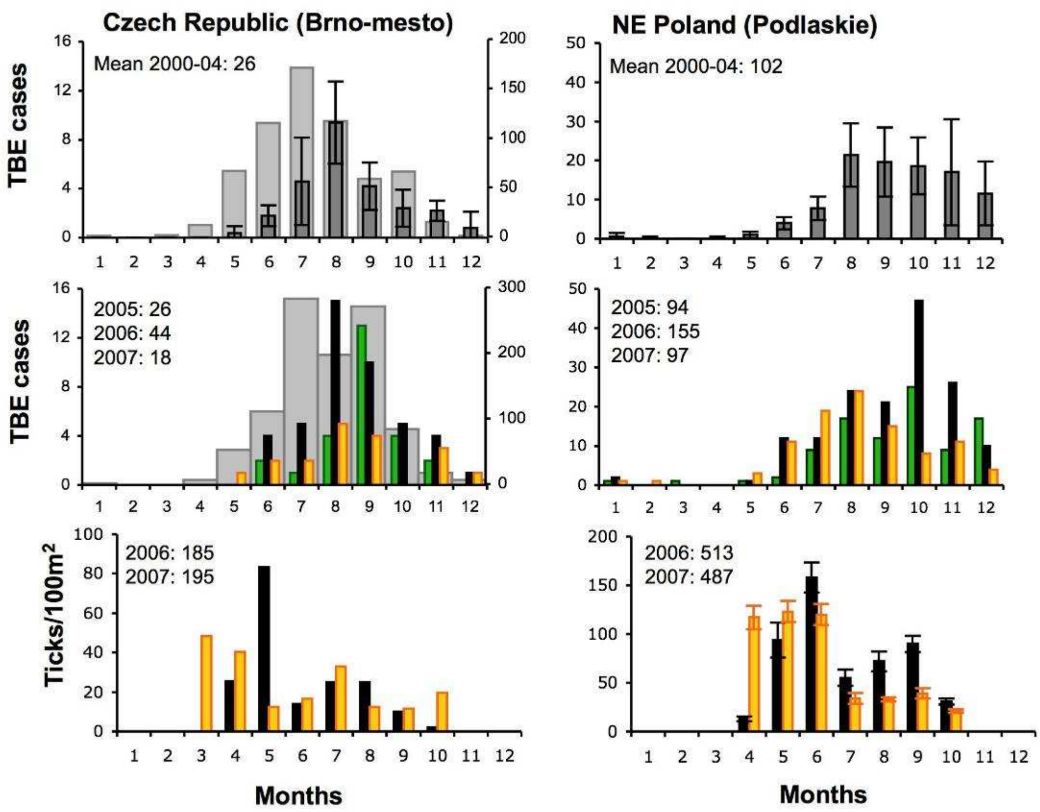

Fig TBD05**:** Monthly distributions of cases of TBE and monthly densities of ticks at Brno-mesto, SE Czechland, and Podlaskie, NE Poland. Upper and middle histograms for each country: means (+ 1 st dev) over 2000-04 (grey), 2005 (green), 2006 (black) and 2007 (golden). TBE case numbers for all Czechland for 2000-04 (mean annual total 101) and for 2006 (total 259) are shown as pale grey bars and on the right-hand *y*-axis, behind the data for Brno-mesto. Lower histogram for each country, mean (± 1 st dev) monthly densities of ticks at 6 sampling sites in Podlaskie and the single sampling site at Brno-mesto, matched to the above TBE incidence areas over 2005 (green), 2006 (black) and 2007 (gold). The tick data are advanced by one month relative to the TBE data to account for the approximate delay between tick bite and TBE registration. Annual total numbers of TBE cases and counted ticks for each year are shown.

A risk map for TBE in Hungary is currently being prepared (ZOOX) in collaboration with HIT-LRRS, based on known human TBE cases in each village from 1970 to 2004. This is being used to explore the shifting distribution of TBE over this period and thus to search for environmental correlates.

Deliverables

D TBD 20 – Time series and multi-variate regression analyses to establish correlations, and possible causality, between the epidemiological, environmental and sociological data from WP1-4 (M48). Achieved at the international level for an increasing number of countries in western, central and eastern Europe within EDEN-TBD, and at the regional level in Italy. 4 new publications (EDEN publication numbers 0073, 0100, 0109, 0112).

D TBD 25 – TBE risk maps for various countries (M54). Under way for Hungary. Level of achievement, 20%.

Milestones and expected result

M TBD 10 - Definition of structure and components of database appropriate for the nature of the information (quantitative, qualitative, discrete and continuous) (M52). Complexity increasingly recognized, solutions still under development.

Workpackages ROBO 1 to ROBO 5: Rodent borne diseases

Deliverable review

Work package	Deliverable number	Deliverable description	Programmed delivery month	Effective delivery month	CEA 18 Italy	IMI 23 Slovakia	METLA 28 Finland	CBGP 29 France	SMI 30 Sweden	UA 31 Belgium	UH.HIA 32 Finland	ULIV 33 UK
WP 1	D-ROBO-01	Pan-European regional (1)	60		B	NR	B	NR	NR	B	NR	NR
WP 1	D-ROBO-02	Field site images	52		A	A	A	A	C	A	NR	A
WP 1	D-ROBO-03	Regional masting (2)	30		C	NR	NR	NR	NR	C	NR	NR
WP 1	D-ROBO-04	Fragmentation	30		NR	NR	C	NR	NR	C	NR	C
WP 1	D-ROBO-05	Snow trends	26		B	NR	B	NR	NR	NR	NR	NR
WP 1	D-ROBO-06	HR landscape change	18									
WP3	D-ROBO-07	Stand. Human diagn (3)	24		NR	C	NR	NR	C	NR	C	NR
WP3	D-ROBO-09	2nd gen. Human robo data	18		NR	NR	NR	NR	C	NR	A,C	NR
WP4	D-ROBO-10	Stand. Virus and rodent molec (5)	30		NR	A	C	NR	A	NR	A,C	A
WP4	D-ROBO-13	2nd gen. Rodent and virus data	30		C	C	C	C	C	C	C	C
W4	D-ROBO-14	1st GIS analyses	30		C	NR	C	A	NR	A,C	NR	C
WP4	D-ROBO-17	MHC genetics	30		NR	NR	C	C	NR	C	NR	NR
WP5	D-ROBO-18	Afforestation	20		NR	NR	C	NR	NR	NR	NR	C
WP5	D-ROBO-19	Robo documents	17		NR	NR	C	C	NR	C	C	NR

	LEGEND		
A	Completed	1	Cannot be obtained for masting at paneuropean scale, but locally
B	Well in progress	2	Delayed due to the HITs
C	Excellent progress	3	Classic methods OK, new microarrays being completed
D	Started	4	Partner shows no interest in this
E	Not done yet	5	Classic methods OK, microarrays being completed
NR	Not relevant	6	Univ Antwerp did not grant relevant animal exp permits
		7	This joint plan with TBE was delayed by a year due to local problems in Slovakia

Backgroun
Involved
NA

Table ROBO-01. Deliverable status per ROBO partner

Executive summary

The work on animal reservoirs and viruses has progressed well.

We have a clear picture of phylogeography of important hantavirus carrier hosts and their hantaviruses. In addition, research on the MHC-genetics, possibly predisposing rodents to hanta infection has produced interesting results. So far, in discussion of the different human hanta (and other robo disease) dynamics in Europe, we have concentrated on different host dynamics and landscapes. Now we have to seriously consider an additional third factor, that MHC genetics of rodents may affect their susceptibility to robo viruses. MHC genetics are incongruent with traditional mtDNA phylogeographies.

A detailed study on the on S and M segments of Puumala virus (PUUV) in a highly endemic region in Finland went on and showed surprisingly large genetic variation and also reassortment at a local scale We have continued this study by following the abundance and "selection survival" of reassortments from the rodent peak of 2005 down and through the decline in 2005-06 and again to a new peak in 2008-08. We have found the contact zone between N Scandinavian and Eastern PUUV lineages in NW Finland, where all kinds of reassortments have been found.

We have new data on the abundance and distribution of LCMV- type viruses in Europe, and we predict that there are several LCVM- type stains in Europe. We have tried to develop methods to recover the LCMV strains but have not yet been successful. Ecological studies on cowpox have progressed well, and sequencing of cowpox strains has started.

Annual/biannual monitorings are running in several countries. Intensive longitudinal monitoring on host dynamics and robo transmission dynamics continued on three selected regions. In N Italy we completed the first long term data set ever on LCMV dynamics in wild rodents, and showed that masting events impact also LCM dynamics.

In Belgium, small scale climate differences seemed to play a role in PUUV occurrence, vegetation index between years, but the abundance of bank voles does not seem to affect the degree of PUUV seroprevalence further. We found indications for a dilution effect on PUUV prevalence, dependent on the relative proportion of non-host wood mice *Apodemus sylvaticus* in a study site.

We regard the combination of a dilution effect, a possible threshold density that depends on local conditions and a higher fragmentation of suitable bank vole habitat in our study area as plausible explanations for the sparse occurrence of PUUV infection and low prevalence detected. In contrast, in Finland we showed that fragmentation due to intensive forestry does not affect the abundance and ubiquitous occurrence of bank voles and PUUV in northern forest landscapes. Thus, strongly cyclic dynamics and extensive "homogenous" landscapes, and absence of dilution species explain the commonness of PUUV in the North. Due to this, PUUV infections in humans start to appear with only a lag of 2-3 months after rodents start to increase. We have a lot of new info of the role host population structure on robo transmission, prevalences and dynamics in various population subgroups, both in Belgian, Finnish and Italian situations.
We started a joint project with TBE team in Latvia to analyse the impact of habitat change (reforestation and afforestation of former agricultural land) on the occurrence of ROBO and TBE risk.
Experimental work on the kinetics of hanta infection process in rodent hosts showed the major virus output early in the chronic infection. We have subsequently expanded this study to wild rodent populations too see if the results are general, and most of all, if maturation and hormonal changes in the hosts can cause long-term variations in virus shedding. Even if the infection is chronic in rodents, it is essential to know for modelling if the virus shedding is only temporal and short term.
Modelling on masting has produced clear results suggesting that summer temperatures are decisive, and in fact, the outbreak in 2005 in western and central Europe could this way be traced back to the heat wave in 2003. It is important to realize that the processes leading to rodent and robo disease outbreaks are long term processes, because too often, especially in the medical circles without ecological understanding, only the simultaneous climatic events are considered. The Finnish data on rodent cycles and NE local dynamics is being compiled, and the Swedish data on last outbreak has been completed..
The available European human data on HFRS has been compiled, and the diagnostic methods in hantavirus research have been updated and reviewed, including the further development of microarray methods.

The Belgian and Swedish human HFRS data have been and is being analysed together with HR and LR HITs, and Slovenian and Finnish human data are at LR HIT. Finnish local vole and virus data are being analysed at HR HIT. Large human serosamples from Italy and Switzerland have been screened. Ecological niche modelling has been started. The aim of the study is relating environmental features to occurrence of human PUUV infection on a large geographical scale within West/Eastern Europe using new modelling techniques. After a delay, mathematical modelling of the robo dynamics in Belgium, Finland and Italy has started.
At the annual meeting of year 3 in Brno, all partners and participants in the Robo group hand an extensive review on their work, and several PhD students participated in the PhD meeting. Quite a number of EDEN and EDEN related papers have been published, and ROBO team members have participated in several congresses.

Publications

The following PhD students have completed their thesis at least partially using EDEN data:

Saksida, Ana, IMI 23. 2008. The role of immune mechanisms and viral load in the pathogenesis of Bunyavirus hemorrhagic fever. Doctoral thesis. University of Ljubljana, Medical Faculty, 2008 (no eden number applied).

Hardestam, Jonas, SMI 30, 2008. Hantaviruses – shedding, stability and induction of apostosis, Karolinska Institutet, Stockholm. (no eden number applied)

Deffontaine, V. CBGP 29. 2008. Evolutionary history of bank vole (Myodes glareolus) in Eurasia. Univ Liege. EDEN0092

The following PhD students are currently conducting research using EDEN data:

- Tersago, Katrien. UA 31 . Environmental attributes of the occurrence of PUUV and HFRS in Belgium

- Voutilainen, Liina, METLA 28, Transmission dynamics and landscape ecology of Puumala hantavirus in N Europe
- Guivier, Emmanuel, CBGP 29, Impact of rodent immune gene diversity on the occurrence of Puumala hantavirus in the bank vole *Myodes glareolus*. Consequence for the geographic distribution and emergence of HFRS.
- Lukomski, Lukasz, ULIV 33, Spatial Aspects of Cowpox in wild rodent populations in Northern England.
- Razzauti, Maria, METLA 28 + UH.HI 31: Reassortment genetics and recombination of Puumala hantavirus in cyclic bank vole populations.
- Malin Stoltz, SMI 30: Molecular biology of hantaviruses
- Karin Sundströmm SMI 30, Molecular biology of hantaviruses
- Kinnunen, Paula UH.HI 32: Genetics and strain variation of cowpox and Borna viruses
- PhD students currently working on EDEN-related molecular biology on hantaviruses at UH.HI 31: Agne Alminaite, Jussi Hepojoki, Paula Kinnunen, Kirsi Moilanen, Tomas Strandin, Hao Wang,

The following scientific documents have been published by EDEN-ROBO team members. Those marked by an EDEN publication number were officially validated by the EDEN Steering Committee, those marked by EDENXXX were supported at least partially by EDEN funds but were submitted for validation to the EDEN SC after publication (no EDEN number included in final paper).

Publications of EDEN-ROBO (with an EDEN number)

CHARBONNEL N, CHAVAL Y, BERTHIER K, DETER J, MORAND S, PALME R, & COSSON, JF 2008. Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. - *Physiological and Biochemical Zoology* 81:63–73. EDEN 0007

BRYJA J, CHARBONNEL N, BERTHIER K, GALAN M, & COSSON JF 2007. Density-related changes in selection pattern on major histocompatibility complex genes in fluctuating populations of voles. - *Molecular Ecology* 16:5084–5097. EDEN0033

CHARBONNEL N, DETER J, CHAVAL Y, LAAKONEN J, HENTTONEN H, VOUTILAINEN L, MORAND S, & COSSON JF. 2008. Serological detection and risk assessment of three emerging rodent borne viral zoonoses in French populations of the fossorial water vole Arvicola terrestris shermani. - *Vector-Borne Zoonotic Diseases* 8:763-768. EDEN0037

TERSAGO K., SCHREURS A., LINARD C., VERHAGEN R. , VAN DONGEN S. & LEIRS H. 2008. Population, environmental and community effects on local bank vole (Myodes glareolus) Puumala virus infection in an area with low human incidence. - *Vector - Borne and Zoonotic Diseases* 8: 235-244. EDEN 0042

DETER J, BRYJA J, CHAVAL Y, GALAN M, HENTTONEN H, LAAKKONEN J, VOUTILAINEN L, VAPALAHTI O, VAHERI A, RIBAS SALVADOR A, MORAND S, COSSON JF, & CHARBONNEL N. 2008. Association between the DQA MHC class II gene and Puumala virus infection in Myodes glareolus, the bank vole. - *Infection, Genetics and Evolution* 8:450–458. EDEN0044

RAZZAUTI, M., PLYUSNINA, A., HENTTONEN, H. & PLYUSNIN, A. 2008. Accumulation of point mutations and reassortment of genomic RNA segments are involved in the microevolution of Puumala hantavirus in a bank vole (Myodes glareolus) population - *J. Gen. Virol.* 89:1649-1660. EDEN 0049

LINARD C, TERSAGO K, LEIRS H, & LAMBIN EF, 2007. Environmental conditions and Puumala virus transmission in Belgium. - *Int. J. Health Geography* 6:55. EDEN 0060

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HEYMAN, P. & VAHERI, A. & ENIVD members. Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006. - *Euro Surveill.* 2008 Jul 10; 13(28). pii: 18925. EDEN 0074

JOHANSSON, P., OLSSON, G.E., LOW, H.-T., BUCHT, G., AHLM, C., JUTO, P. & ELGH F. 2008. Puumala hantavirus genetic variability in an endemic region (Northern Sweden). - *Infection, Genetics and Evolution* 8: 286-296. EDEN 0075.

VAHERI, A., VAPALAHTI, O. & PLYUSNIN, A. 2008. How to diagnose hantavirus infections and detect them in rodents and insectivores. - *Rev. Med. Virol*. 18:277-288. EDEN 0078

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DEFFONTAINE, V., LIBOIS, R., & MICHAUX, J.R. Endemism and different phylogeographic histories of bank voles Mediterranean lineages. - MS to be submitted, EDEN 0053

HARDESTAM, J., PETTERSON, L., AHLM, C., EVANDER, M., LUNDKVIST, Å. & KLINGSTRÖM, J. 2009. Antiviral effect of human saliva against hantavirus. - *Microbes and Infection.* (in press) EDEN 0082

OLSSON, G.E., HJERTQVIST, M., LUNDKVIST, Å. & HÖRNFELDT, B. 2009. Predicting high risk of human hantavirus infections, Sweden. - *Emerg Infect Dis* 15 (1). [Epub ahead of print] EDEN 0096

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Meetings where papers involving EDEN-ROBO results/ ideas have been presented

- 16th Meeting of EU group on "European Network for Diagnostics of Imported Viral Diseases", Limassol, Cyprus; A Vaheri was Chairman of Session on Hantaviruses and gave 2 presentations on hantaviruses.
- 5th International Conference on Emerging Zoonoses, Limassol, Cyprus; A Vaheri gave a presentation on hantaviruses.
- 17th Meeting of EU Group on "European Network for Diagnostics of Imported Viral Diseases" and Iberoamerican Netwowrk on Emerging Viral Diseases, Madrid, Spain; A Vaheri was Chairman of Session on Hantaviruses and gave a presentation on hantaviruses.

- 14th International Congress of Virology, Istanbul, Turkey; A Vaheri was Chairman of Session on hantaviruses.
- FAO, European Forest Week, Rome, Session on Forests and Health. H. Henttonen, Zoonotic diseases from European forests
- Specialist Consultation on Emerging Diseases in South-East Asia (SCEDSEA): Bridging science and society for a healthy future Bangkok, Thailand. S. Morand Environmental changes and host-pathogens distribution patterns
- Institute for Animal Health in Pirbright, L. Lukomski: Spatial Aspects of Cowpox in wild rodent populations in Northern England.
- Annual meeting of the British Ecological Society, L. Lukomski; Spatial aspects of Cowpox in wild rodent populations in Northern England.
- Stockholm County Council, Department of Communicable Disease Control and Prevention, Stockholm, Å. Lundkvist, Klimatförändringar & infektionssjukdomar [Climate change & infectious diseases].
- ECDC, Stockholm, Å. Lundkvist, GIS as a tool in public health.Congress for The Swedish Association of Environmental Health Professionals, Stockholm, Å. Lundkvist, Klimatförändringar & infektionssjukdomar [Climate change & infectious diseases].
- ECDC Workshop participation, Stockholm, Å.: Lundkvist. Linking Environmental and Infectious Diseases Data.
- SIDA Workshop participation, Stockholm, , Å. LundkvistHuman Health and Forests.
- ESA/ESTEC Workshop participation, The Netherlands, Å Lundkvist,.; Tick-borne encephalities risk mapping.
- ECDC Workshop participation, Stockholm, Å Lundkvist,. : Road Map for Implementation of a European Environment and Epidemiology (E3) Network.
- DG SANCO workshops participation (1) Initiative for Generating Operational Risk Maps for Communicable Diseases using Integrated Space and Non-space Assets (2) The Consequences of Climate Change on Human and Animal Health. H. Henttonen and G.Olsson
- ENIVD network participation: T. Avsic-Zupanc, Å. Lundkvist, O.. Vapalahti, A.Vaheri
- COHAB- 2nd Int. Conf. onHhealth and Biodiversity, H Henttonen: Occurrence and dynamics of rodent-borne viruses in Europe (invited talk).
- ECDC, Stockholm – V-borne meeting. H. Henttonen, H Leirs & A Rizzoli
- Network of Climate Change Risk on Forests, Umeå, Sweden, H Henttonen, The changing patterns of vole fluctuations in N Fennoscandia
- 10th Nordic Viral Zoonoses Network Workshop, Seili, Finland: Henttonen, Lundkvist, Olsson, Vaheri, Vapalahti, Voutilainen gave talks
- Annual EDEN meeting in Brno, January 2008: ROBO participants: Avsic-Zupanc, Charbonnel, Cosson, Crespin, Durmisi, Guivier, Henttonen, Knap, Leirs, Lukomski, Lundkvist, Olsson, Rizzoli, Tagliapietra, Tersago, Vaheri, Voutilainen,
- 7th Baltic Theriological Congress, Lepanina, Estonia:
    - Michaux, J. Phylogeographic history of North European mammals: implication on their evolutionary history and their conservation. (Invited plenary talk)
    - Henttonen, H. Rodent-borne viral Zoonoses in Europe. (Invited plenary talk)
- 11th Rodents et Spatium (International congress on rodent biology), Myshkin, Russia (talks given)
    - Henttonen, H. Rodent-borne viruses in N Palearctic
    - Henttonen, H. Rodent dynamics in N Finland
    - Voutilainen, L. Transmission dynamics of Puumala hantavirus in the northern taiga
    - Deffontaine, V. Phylogeography of the bank vole (*Myodes glareolus*) in Eurasia and its implications for the study of Puumala virus evolution.

Student exchanges in ROBO

- Emmanuell Guivier (CBGP) visited Helsinki (METLA & UH.HI) 9.6.-14.8. 2008
- Lukasz Lukomski (ULIV) spent one week in Louvain with Lambin team in February 2008.

WP ROBO 1 – Landscapes, biotopes and habitats

Work package number	Robo1	Start date or starting event:				37	
Participant id	METLA	UA	CEA				Total
Person-months / participant	6	3	1				10

Objectives

The overall objective of this WP is to understand patterns and processes linked to climate and the environment which (may) have an impact on, and will be related to the regional rodent/virus diversity and population dynamics in Europe.

Work performed during previous reporting periods

A list of working hypotheses was drawn of the climate and environmental parameters to be included in the regional spatial databases and models. Data compilation in progress on masting, forest resources and snow cover (Metla, UA, CEA) with assistance from the HIT data management team (EuroAegis). High resolution land-use and land-cover satellite imagery for each field site is in process with assistance from the HIT high resolution remote sensing (UCL). Data compilation for two main study regions, Belgium and Finland for a regional model on forest fragmentation patterns is proceeding.

The Finnish and Slovenian human data set sent to LR HIT. Belgian results on environmental correlated to PUUV published. Swedish LR work on human data goes on. UK cowpox work with environmental correlates started. The compilation of pan-European environmental data continues. A list of working hypotheses was drawn of the climate and environmental parameters to be included in the regional spatial databases and models.

Description of work

Both Pan-European and local spatial analysis studies will be conducted in collaboration with the relevant horizontal integration teams.

A – Pan-European spatial analysis:

1 – Masting dynamics: Climatic data will be collated on a regional (remotely sensed – RS) and country (ground measured – GM) basis because temperature induced masting is a large-scale phenomenon. Standard regional RS 1-8km resolution meteorological and vegetation data will be made available through the common EDEN data archive. GM meteorological data will be collated by the resident teams of each country participating to the ROBO sub-project. Historic and current day data on masting will be collated from various literature and archive/www sources. Masting dynamics will be analyzed regionally in relation to climatic factors. Particular attention will be given to assessing the scale of geographic synchrony in masting and the identification of trends (UA).

Achieved results: *Temporal analysis: effects of tree seed production and climate*

Recently, human case numbers of Puumala virus infection, nephropathia epidemica (NE), in Europe are increasing. Explanations for this altered epidemiology must be sought in factors that cause reservoir host, bank vole (*Myodes glareolus*), abundance peaks, as high bank vole numbers induce higher transmission rates towards humans. In temperate Europe, these abundance peaks are often related to tree masting, which is supposedly triggered by specific weather conditions. UA evaluated the relationship between tree seed production, climate and NE incidence in Belgium and show that NE epidemics are indeed preceded by abundant tree seed production.

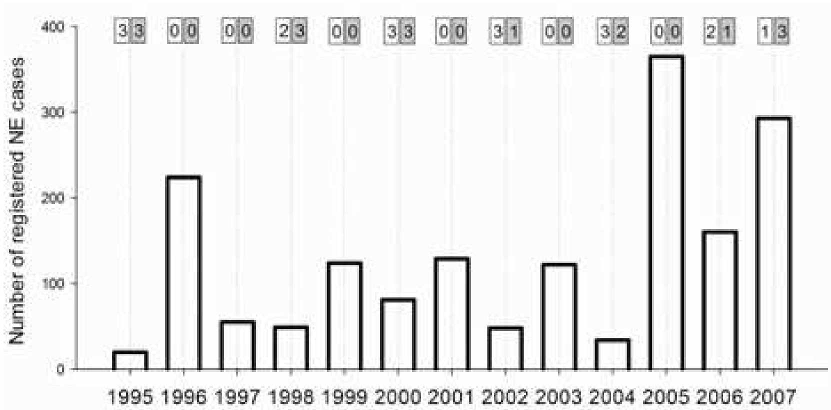


Fig. ROBO01 - Annual number of NE cases per year and the category of seed production in the whole Southern Belgium territory of beech, F. sylvatica (white) and native oak, Q. robur and Q. petraea (grey) in the respective year. Categories of fructification are ordered from 0 = Low, 1 = moderate, 2 = good to 3 = very good.

Also a direct link between climate and NE incidence is found. High summer and autumn temperatures, respectively two years and one year before NE occurrence, relate to high NE incidence (Fig 02). This enables early forecasting of NE outbreaks. Since future climate change scenarios predict higher temperatures in Europe, we should regard Puumala virus as an increasing health threat.

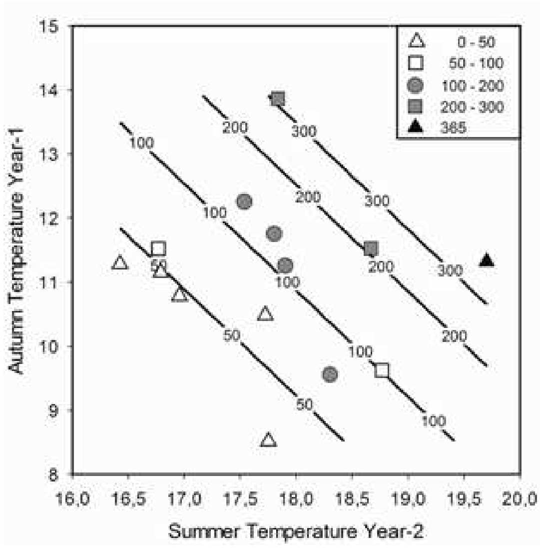


Fig. ROBO02 - Contour plot of the yearly number of NE cases against the average summer temperature (°C) 2 years before and average autumn temperature (°C) one year before NE infection. Contours are based on the selected statistic model. Intervals of raw NE data are shown in the legend.

These results were published; Tersago K. et al. 2009 Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. Epidemiology and infection. (in press) - doi:10.1017/S0950268808000940 (EDEN 0097).

Beech seeds production in Trentino: we continued the evaluation of the production of seeds in a thermophilic beech woodland (*Fagus sylvaticus*). The study site is located in Dos Gaggio, where the intensive rodent monitoring is performed. This year production was very low. Since the year we started the situation is the following:

YEAR	TOT. N° SEEDS
2005	2
2006	657
2007	2959
2008	**25**

2 – Forest resources: Existing archives of forest resources and landscape structure will be used: tree species, homogeneity, age, patchiness-dynamics, trend analysis. This is relevant to countries with recent great changes in agriculture.

Achieved results: Distributions and abundance of beech and oak have been preliminarily compared in an effort to understand the phase difference in human hanta outbreak in 2005-06 in Germany, with idea that beech and oak could have a one year phase difference in their masting

3 – Regional snow-cover extent and duration trends will be analysed in relation with vegetation zones.

Achieved results: Snow cover maps (CEALP)
The "Global EASE-Grid 8-day Blended SSM/I and MODIS Snow Cover" raster maps (spatial resolution: 0.28 degree, approx. 25 km pixel length) were reprojected from the EASE-Northern hemisphere projection (LAEA based) to longitude-latitude graticule. This data set is a 8-day snow-covered area (SCA) and snow water equivalent (SWE) data set and available from 1/2000 onwards. The Global SWE data are derived from the Special Sensor Microwave Imager (SSM/I) and were furthermore enhanced with MODIS/Terra Snow Cover 8-Day Level 3 Global 0.05 degree Climate Modeling Grid (CMG) data by NSIDC. These data are suitable for continental-scale time-series studies of snow cover and snow water equivalent. Since the data became available only in November 2008 in readable form, first data analysis is anticipated in the next weeks.

4 – High resolution local studies in Finland, Belgium, France and Italy: environmental correlates with host and virus occurrence

Achieved results: HR maps and related data have been used in detailed analyses of host and virus occurrence and dynamics (see other WP's)

B – LR studies: Environmental background in long term human Finland.

Achieved results: The human data are in Oxford.

Deliverables
D Robo-01: Pan-European regional (1-8km raster data and vector data) spatial data base on masting, forest resources and snow cover (M36). No Par-European maps, local based on case studies D Robo-02: Processed high resolution land-use and land-cover satellite imagery for each field site (M36) Completed for most sites. D Robo-03: Regional models on masting dynamics (M36). Done. D Robo-04: Regional models on forest fragmentation patterns (M36). Have been achieved in the connection of local studies D Robo-05: Regional model on snow cover trends (M36). Not done yet, in progress D Robo-06: Supervised local high resolution landscape change models for selected study sites where relevant changes have occurred, (e.g., Belgium, southern France) (M30). Latvia study site, to be done
Milestones and expected result
M Robo-03 - Spatial data layers are used to generate spatial analysis models for remaining countries (M36). In progress M Robo-04 - Model outputs are available and allow for (i) multi-factorial spatial analysis on virus/rodent dynamics and (ii) extrapolation to other parts of Europe at later stage of project (WP5) (M36). In progress M ROBO-11 - Environmental data used for understanding the distribution of rodent-borne viruses and their hosts as well as their dynamics and epidemiology (M36). In progress

WP ROBO 2 – Vector bionomics and competence

Not relevant

WP ROBO 3 – Public health and human activities

Work package number	Robo3	Start date or starting event:			37	
Participant id	CEA	IMI	CBGP	SMI	UH.HI	ULIV	Total
Person-months / participant	0	8	1	21	15	0	45

Objectives

The general objective of this WP is to describe and analyse the emergence patterns of diseases caused by hanta- and other roboviruses in Europe and adjacent regions.

Work performed during previous reporting periods

Standardised human diagnostics protocols have been discussed in detail at KOM and subsequently, and are operational in all main human labs (UH.HI, SMI, IMI, and UA). In addition, student and sample exchanges between labs have been conducted. New microarrays being developed in parallel at UH-HI, (though not a deliverable but important for future sample processing in greater quantities). The existing data and general pattern of hantaviruses diseases known but there are still white spots especially in E Europe. Excellent data from some countries like Finland, Slovenia, Belgium, Sweden. New large materials from Italy and Switzerland have been analysed at SMI. Human disease data by LCMV complex is fragmentary, some data from Finland, Croatia, northern Italy, UK exists, and they seem to show that in some regions at least in southern Europe LCMV antibodies are more common in humans than hanta antibodies. Generally LCMV seropositivity is around 3-5% which can be considered a surprising result. A careful risk study by UH.HI showed that important risk factors w ere being a male, farmer and smoker.

EDEN partners have screened materials from Italy, Switzerland, Hungary, and Croatian and the Slovenian forestry workers. The available historical European human data on hantavirus diseases, caused by PUUV DOBV and SAAV, have been compiled and will be published soon

Description of work

New human samples from poorly studied regions will be gathered (UH-HI, SMI).

Achieved results:

- SMI has analysed 2,000 human samples from Switzerland and 3,000 from Italy.
- A compilation of hantavirus human cases in Europe has been published by Heyman & Vaheri (2008), UH.HI
- SMI has studied the presence of viral RNA in patient saliva and shedding of virus in human saliva. We have further studied the importance of saliva for the transmission of hantaviruses among the rodent reservoir.

Detailed analyses of 2007-08 human material from observed peaks will be performed in Finland, Sweden and Belgium (UA, UH.HI, SMI).

Achieved results:

- Swedish data partly published,
- Finnish data locally (Central Finland) analysed (See P 4), national analyses in progress,
- Belgian data being analysed (see WP5).
- Warm late autumns and early winters postpone the epidemics to start later, and there have been a lot of cases still in January – March.

The in depth analysis on the Finnish human data will continue (UH.HI, METLA, HIT-LR). Further development of microarrays will be performed.

Achieved results:

- The detailed analysis of the Finnish human data 1995 onwards is being analysed, and a manuscript is being completed.
- The record human peak in early 2008 in Sweden has been analysed (Olsson et al. 2009). The epidemic did not occur in the normal period in late autumn, probably due to the warm weather, but after the cold spell in January, new human record took place.

- The work on microarray technology has continued (Moilanen, EDEN-related papers).
- A review of hantaviral diagnostic methods has been published (Vaheri et al 2008, EDEN 0078)
- Due to the all-time rodent peak in the southern half of Finland (based on the national vole survey by METLA, the densities in many locations are highest in 20 – 30 years), the new record in human NE cases will be achieved in 2008 (figure below). So, far two fatal cases have been recorded this year. The other one is being studies in great detail (see WP 4, reassortment).

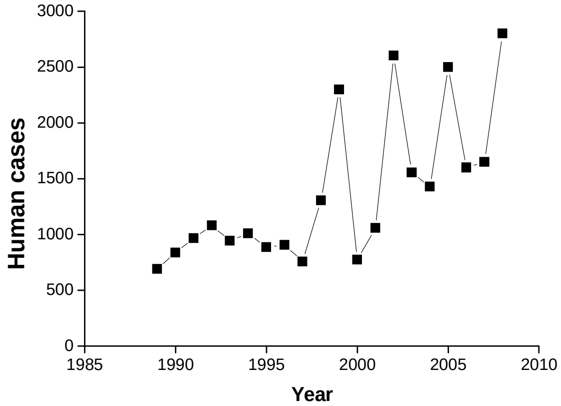
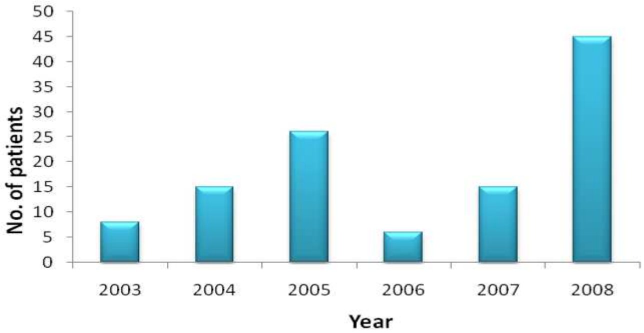


Fig. ROBO03 - NE dynamics in Finland. The 2008 number is from early December, and the final number for 2008 may increase over 3,000. The previous all time record was 2,600 cases in 2002. However, the warm early winter in 2005 delayed the epidemic and in Jan. and Feb? 2006 still over 700 cases occurred. Same may happen this winter, too.

Based on very promising results published for Belgium in the previous reporting period the relationship between masting years and human NE dynamics will be further studied in Western Europe.

Achieved results: The Belgian results of temperatures, masting and human NDE dynamics, and the model have been published (see WP 1).

The year 2008 was a peak year for human HFRS in Slovenia (figure below). Interestingly, based on northern Italian (CEALP) masting data, 2007 was a mast year. Still, in Trentino rodents were low in 2008, maybe due to very low seed crop then. However, it could be supposed that masting is synchronous in northern Italy and Slovenia, thereby possibly explaining the human peak in Slovenia in 2008.



Deliverables

D Robo-07. Standardised human diagnostics protocols and new microarrays ready for human diagnostic (M36). EDEN 0078

D Robo-09. Updates of the second generation maps of observed human rodent-borne virus presence and serological prevalence in Europe (M36). EDEN 0074, EDEN applied

Milestones and expected result
M ROBO-06 - Updated maps of observed human rodent-borne virus presence in Europe (M36) EDEN 74
M ROBO-07 – Serological data, especially those from groups at highest risk, contribute to further target work in WP 4 (M25-42). Human data and cases have been used to focus rodent work

WP ROBO 4 – Animal reservoirs

Work package number	Robo4	Start date or starting event:				37	
Participant id	METLA	UH.HI	SMI	ULIV	UA	CBGP	CEA
Person-months / participant	29	30	33	15	20	27	8
Participant id	IMI						Total
Person-months / participant	7						169

Objectives

The general objective of this WP is to understand and quantify the role of the rodent reservoir in the distribution and transmission of identified emerging robo diseases. The specific objectives during the next 18 months are:

- To further train local teams for rodentological and virological sampling for field/disease surveillance and monitoring; at this stage this concerns candidate countries and adjacent regions particularly.
- To improve understanding and knowledge of the distribution of rodent reservoir species and the robovirus species they harbour in Europe, and establish their phylogeography and the importance of phylogeographic contact zones.
- To further reinforce reasonable network of local study sites for sampling for various purposes and representative monitoring.
- To test how environmental factors affect the virus persistence in different environmental conditions outside the host, which is critical for modelling and control of human infections.
- To understand the robo virus transmisson and density-dependence of robo dynamics in the host populations
- To understand the virus shedding dynamics and kinetics in the host during the infection
- To understand factors affecting temporal MHC-variation in hosts in relation robo dynamics, and to understand the dynamics of genetic variation and reassortment also at the local scale

Work performed during previous reporting periods

The presence or absence of hantaviral diseases is now rather well-known for many areas (but not all). Presence of arenaviruses (LCMV "complex"), cowpox and Borna is more fragmentally known. On the other hand, seasonal and long-term hantaviral dynamics are reasonably well-known only for selected countries, but for the rest of robos, dynamical aspects are so far poorly known. While the basic patterns of hanta dynamics are known (masting induced outbreaks in temperate zone and predation driven vole cycles in boreal forests), the contrast holds for arenas.

Various go and hit, annual/biannual and intensive trapping programs continued. Kinetics and shedding of PUUV were studies. The cyt-b and MhC phylogeography of the bank vole were analysed. New PUUV lineages were analysed. Details of transmission dynamics of PUUV both in cyclic populations in the north and mast driven populations in Belgium were studied, and modelling started. Cowpox dynamics and environmental correlated studies in the UK. The role of MHC in hantavirus risks for rodents were analysed, and the role of kinship in hantavirus dispersal was found.

Description of work

Various trapping programs will continue and results analysed in relation to density, region and environmental parameters (METLA, SMI, UA, GBGP, ULIV, CEA). Special attention will be given to the role of various subgroups (functional groups) in rodent populations (METLA). 2008 will be a peak year in Finland and also probably in N Sweden. Results of the Belgian peak in 2007 will be analysed (UA).

Achieved results:

General

Saaremaa hantavirus has been accepted by International Committee on Virus Taxonomy as a unique virus. This virus was first found by METLA and UH.HI teams, but its status as a separate virus, not as a lineage of Dobrava, has been debated.

Monitoring of PUUV infection in Northern Belgium (low incidence area)

The distribution of PUUV infection in local bank vole *Myodes glareolus* populations in an area with low human PUUV infection (NE) incidence in Northern Belgium was monitored for two consecutive years (2004-2005) and then analysed in 2006. Bank voles were trapped in preferred habitat and tested for anti-PUUV IgG. Infection data were related to individual bank vole features, population demography and environmental variables. Rare occurrence of PUUV infection was found and PUUV prevalence was low compared to data from the high NE incidence area in southern Belgium (Fig ROBO04).

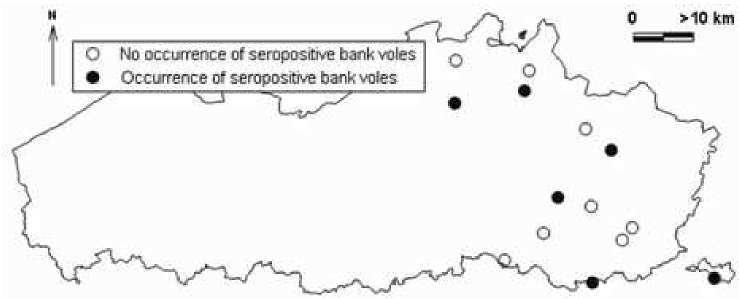


Fig. ROBO04: Occurrence of seropositive bank voles in the selected study sites in the year 2004/2005.

Small scale climate differences seemed to play a role in PUUV occurrence, vegetation index between years, but the abundance of bank voles does not seem to affect the degree of PUUV seroprevalence further. We found indications for a dilution effect on PUUV prevalence, dependent on the relative proportion of non-host wood mice *Apodemus sylvaticus* in a study site (Fig. ROBO05). We regard the combination of a dilution effect, a possible threshold density that depends on local conditions and a higher fragmentation of suitable bank vole habitat in our study area as plausible explanations for the sparse occurrence of PUUV infection and low prevalence detected. Thus, besides human activity patterns, local environmental conditions and rodent community structure are also likely to play a crucial role in determining PUUV infection risk for humans. Tersago K et al 2008 (EDEN 0042).

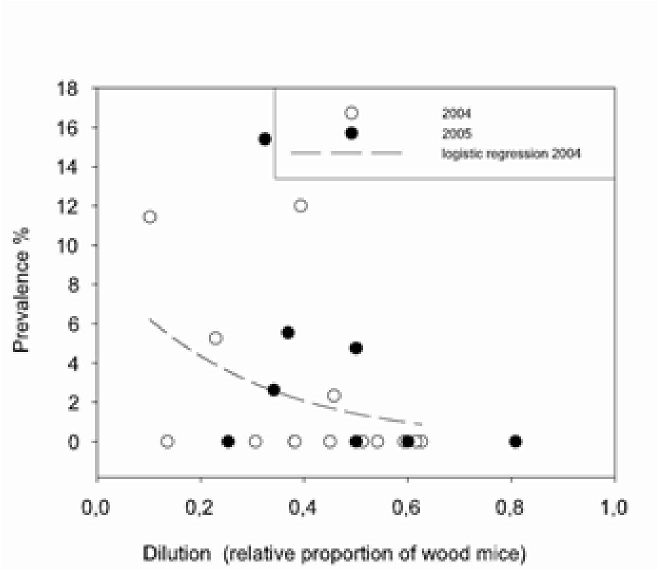


Fig. ROBO05: PUUV prevalence in relation to the relative proportion of wood mice

Additional monitoring in the selected field sites was carried out during summer 2007 (new epidemic year in Belgium). These data allow us to test the hypotheses on PUUV distribution, threshold and dilution effect, formulated earlier. Serum samples were analysed for antibodies against PUUV and Cowpox during the last year. Analyses of these data are under progress.

CMR study in southern Belgium

Based on a 10-year dataset of human NE incidence in Belgium, 9 study sites were selected in communities with different NE incidence patterns. Sites reflect preferred bank vole biotope in public forests. During the years 2004-2005-2006, CMR (Capture-Mark-Recapture) studies were carried out three times a year (spring-summer-fall), enabling monitoring of vole numbers and individual blood sampling (serum IgG detection) in all 9 study sites. Data on bank vole presence, movement, condition and PUUV infection patterns were collected. Environmental features by means of spatial imagery studies, field soil parameters and species inventarisation add additional information to our results.

1. Local reservoir host populations in relation to Puumala Hantavirus infection dynamics

Differences in occurrence of infection were clear in 2004, where only in 5 of 9 sites PUUV IgG positive bank voles were found, enabling us to compare among sites. Yet, in spring 2005 8 of the 9 sites presented PUUV infection. A general increase in prevalence and number of infected animals was found from year 2004 towards spring-summer 2005, these results were reflected by the number of reported NE cases that reached a historical maximum (over 300 cases) in Belgium that year (Institute of Public Health, Belgium). After the peak in 2005, all populations crashed over winter and during spring 2006 in 4 of 9 study sites no animals were found, in the other sites numbers did not exceed 11 MNA/ha. PUUV seroprevalence was found in one study site. Towards fall 2006 a small increase in population numbers was observed, but only in one site a re-emergence of seroprevalent bank voles had occurred.

Detailed population data analyses point out importance of postponed reproductive activity and decreased sex ratio in the period preceding the infection peak. Subsequently, a spatial spread of PUUV infection was detected. The local proportion of males was often positively related to the number of seropositive voles. Density-dependence of local PUUV prevalence and seropositivity was only important during the increasing phase of infection. Therefore, after the peak of vole-to-vole infection, a shift towards a density-independent transmission process is suggested. Maternal antibodies and reduced virus shedding, combined with an early decrease in reproductive behaviour, are suggested as explanations for the sudden reduction in bank vole PUUV infection (Tersago et al., submitted manuscript EDEN 0115).

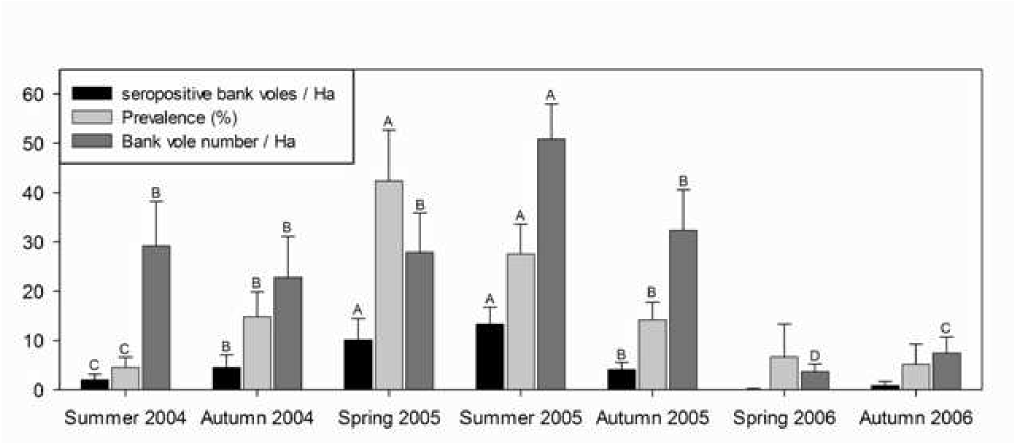


Fig. ROBO06: Regional (all study sites combined) number of PUUV IgG seropositive bank voles / Ha, PUUV IgG prevalence and bank vole number / Ha in each season with respective std errs. Significant seasonal differences for each variable are marked by different letters.

2. Spatio-temporal variation in probability of individual bank vole PUUV infection

Significant differences in probability of PUUV IgG prevalence was found among sexes and age classes with varying sexual condition. Cluster analyses on individual PUUV infection in the field sites are in progress. Genetic relatedness and PUUV IgG seropositivity Microsatellite analysis on tissue of more than 300 bank voles, trapped during our CMR study, was carried out in cooperation with robo-team (CBGP). Extractions (UA), PCR/readings (CBGP) and first analyses are finished. A combined manuscript of all above parameters is under construction at this moment.

3. Cowpox in Belgian bank voles

More than 1,000 bank vole samples were checked for antibodies against cowpox and high prevalence of anti-cowpox IgG was found. Bank vole tissue (lung) sample dissection and additional cowpox virus analyses (PCR) are scheduled. The final analysis and manuscript writing will be carried out during the next year.

4. MMTV

Mouse-mammary tumour virus (MMTV) is a virus that circulates in wild house mice. It is closely related to human mammary-tumour virus and it is believed that it may play a role in the occurrence of some forms of breast cancer in humans. Ford et al. (2003) reported a prevalence in humans of >40% in Australia but <1% in Vietnam and suggested that this could have to with the presence of *Mus domesticus* in Australia, while in Vietnam another species of house mouse, *Mus musculus* is present. Since both species occur in Denmark with separate distribution areas and a narrow hybridisation zone in between, we set up a study to test for the presence of this virus in Danish house mouse populations.

In a laboratory colony of each species, we found no prevalence in *M. musculus* (n=10) but 9/10 positive animals in *M. domesticus*. In wild *Mus domesticus* in and south of hybrid zone the hybrid zone in Denmark, we found 31/39 MMTV-positive animals. Also 2/2 *Mus musculus* from the hybrid zone turned out to be MMTV-positive. Further work is in progress.

A preliminary comparison of the prevalence of human breast cancer in Denmark, showed a higher prevalence in areas where *M.domesticus* is the only or the more prevalent species of house mouse, in comparison to areas where *M.musculus* is more common or *M.domesticus* is absent.

5. PUUV in a metapopulation system

In 2006 rodent trappings were carried out in a well known and documented metapopulation system near our university. This work was done, in order to start a study on the spatial behaviour of PUUV infection in bank vole populations in a patchy landscape. Serum samples were analysed in the beginning of 2007, yet no antibodies against hantavirus were found in the local bank voles.

Field work in Finland

Predicting hanta dynamics and NE 1.

The National vole monitoring and prediction of hantavirus dynamics (and damage in forestry plantations) in Finland has continued. The human peak in 2008 (see WP 3) was well predicted already a year before, however, the magnitude of this rodent peak is unparallel. We predict the peak to crash in spring 2009, and the next peak to occur in 2011. The large geographic synchrony of the rodent peaks in southern and central Finland (where most people live) since 1999 has contributed to the new patterns of human NE dynamics (see WP 3). The peak in 2008 further documents this pattern.

Erreur ! Des objets ne peuvent pas être créés à partir des codes de champs de mise en forme.

Fig. ROBO07: The long term longitudinal CMR study

The intensive monitoring of the transmission dynamics at out long-term study site in a highly endemic region has continued (figure below). As told, 2008 has been a peak year and we have collected a lot of additional materials (next paragraph). The study will be finished this winter with the population crash. The material is being analysed in respect of the role of season, population density, cyclic phase, and the roles of population subgroups in transmission dynamics. These data show that when the bank vole density starts to increase, the Puumala hantavirus follows immediately the host increase (higher part below). Our replicate (satellite) grids at the landsape level (lower part below) indicate that PUUV spreads quickly in the environment. We have found how the different populations structure in the increase and peak phase affect the transmission, and obviously maternal antibodies often slow down transmission in the peak phase.

We have a considerable number of animals for which we know the time of infection and we have been able to monitor these individuals for months, sometimes a year. We have this year sampled animals to study the excretion of virus in natural situations. Earlier SMI team has shown that in lab conditions experimentally infected PUUV is shed only for about 2 months in feces and urine even though the animals are chronically infected – which is very important information for modeling PUUV dynamics. Now, we try to verify or refute this in natural conditions. We can also verify for a number of animals followed for months if their sheddings patterns are constant or change due to the maturation in spring, and if there are differences seasonally and between sexes. The quantitative PCR analyses are being done by SMI team in Stockholm. SMI has further studied in lab the importance of saliva for the transmission of hantaviruses among the rodent reservoir.

Erreur ! Des objets ne peuvent pas être créés à partir des codes de champs de mise en forme.

Fig. ROBO08: Long-term monitoring of PUUF dynamics in bank voles

Erreur ! Des objets ne peuvent pas être créés à partir des codes de champs de mise en forme.

Fig. ROBO09: Spread of PUUV among study grids in a Finnish forest landcape, a metapopulation approach.

In an earlier study, it was found that PUUV survives infectious at least two weeks outside the host. The virus strains that were successful or not in survival were later analysed in detail and it was found that a specific mutation was found to be associated with PUUV transmission from rodent to rodent (SIRONEN, T., KALLIO, E. R., VAHERI, A., LUNDKVIST, Å. & PLYUSNIN, A. 2008. Quasi-species dynamics and fixation of a synonymous mutation in hantavirus transmission. - *J. Gen.Virol.* 89:1308-1313, 2008.)

The impact of forestry in northern landscapes

Forestry affects drastically Finnish and northern European landscapes. It has been evaluated that in every 20 years some 20% of the Finnish forest coverage is cut, i.e. at the moment, about 20 % of forests (= plantations and young stands) are less that 20 years old, another 20% is between 20 and 40 years. The carrier of PUUV the bank vole is known as a habitat generalist in various forest types. Therefore, we tested in a typical landscape of intensive forestry in North – Central Finland, how the bank vole and PUUV are distributed among successional stages, i.e. do young clear cuts increase habitat fragmentation in the eyes of bank voles like agriculture in Central Europe, or is the landscape all the same for bank voles no matter about forestry activities. If the latter case is true, it could contribute to the commonness of PUUV and NE in northern Europe. The design included 6 successional stages (see the figure below), 10 replicates per most successional stages, and 5 small quadrats on each replicate, and trappings were done in early summer and autumn in rodent peak 2007 in that part of Finland .

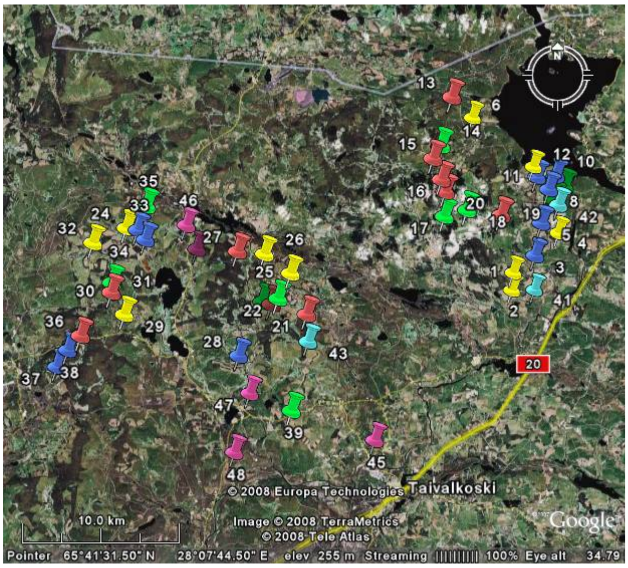


Fig. ROBO10. Locations of study areas : light blue (new clear cuttings), yellow (clear cuts 3-4 years), dark blue (9-11 years), green (24-26 years), red (old forests), pink (old fields)

Seroprevalence of PUUV on various successional stages

Season	Old	1-year	3 – 4	9 – 11	24 – 26
Autumns	19	12	24	21	26
Spring	57	57	25	59	70

Results indicate clearly that in the autumn of the peak year (human epidemic phase) bank voles occur abundantly in all successional stages, and also that PUUV is rather evenly distributed in all forestry landscapes, i.e. the fragmentation due to the forestry does not prevent the spread and commonness of PUUV in the Finnish landscapes. However, it seems also that the overwinter survival of bank voles is highest in old forests compared to early successional stages. Because the autumn – early winter is epidemic season, the lower late winter survival of bank voles in young forest stages does probably not affect much the human risk. Summarizing, the drastic fragmentation of northern forests due intensive forest management, detrimental for many old forests species, does not affect the abundance of the bank vole, carrier of Puumala hantavirus, a generalist forest species.

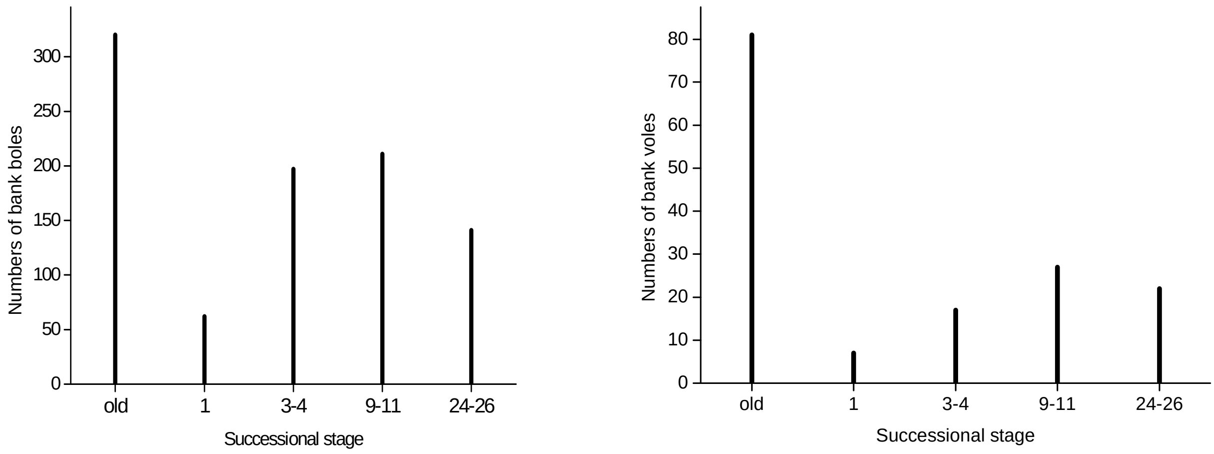


Fig. ROBO11: The abundance of bank voles on different successional stages in autumn (left) and spring (right). (Notice the difference in Y axis).

Habitat change of former agricultural land (ROBO METLA, TBE OX,Latvia & UH.HI)
In large areas in Europe, extensive areas of former agricultural land have abandoned or actively afforested. These habitat changes induce the risk that virus and vector carrying forest rodent species come to closer contact with human settlements and humans. A joint study by EDEN ROBO and TBE teams was stated in 2008 in Latvia.
Nine study areas in western and eastern Latvia were chosen, each area had four habitats, present grain field, fallow, bush and old forests. At each site, a rodent trapping scheme was performed, and ticks were collected. Trappings were done in spring with heavy tick infestation period, and in October.
In spring the densities were rather low, and 241 small mammals were collected. In autumn the densities were higher, and 1,310 small mammals were trapped.
In spring low densities rodents were distributed in their optimal habitats. Serological evidences for several roboviruses (Puumala, Saaremaa, Tula, Dobrava, LCMV, Cowpox) were found, and the viruses seemed to be widely distributed in Latvia. TBE and tick analyses are in progress.
In the autumn, during higher densities, evidence of spill-over habitat selection by forest rodents was found, i.e. forest rodent densities in the bush were considerable, and this can be considered a longer term seasonal occurrence. On the other hand, there were also forest rodents in the fields, but it is known that they come temporarily to field after grain harvesting to feed on falled seeds. Viral data analyses on autumn material are in progress, as well comparison with the tick data. .

Field work in Italy (CEALP)
Longitudinal monitoring
We continued our longitudinal monitoring of a population of *Apodemus flavicollis* in the site of Cavedine initiated in the year 2000. Following our standardized protocol, individual rodents were live trapped in 4 replicated grids of 64 traps each operated fortnightly from April to October, and monthly from November to March. Individuals were uniquely marked with the implantation of a subcutaneous transponder (Trovan® LID100). Biometrics data were collected along with a blood sample. Manipulation and blood sample procedures were approved by the regional wildlife management committee.
We performed 17 trapping sessions from January to November 2008. In total we captured 135 yellow-necked mice, 32 of which were captured and marked in 2007. Sex ratio and the number of sera samples taken for Hanta/Arena/Cowpoxviruses analyses are summarized in table 1.

Table 1

Species	Females	Males	Total	M/F	N° sera samples
Apodemus flavicollis	72	63	135	0.87:1	405

Despite a moderate autumnal peak of animals in 2007, probably affected by the availability of beech seeds in 2007; in 2008 the number of animals was low and after the usual summer peak the population didn't show any autumnal reproduction period. A possible reason could be the scarcity of seeds, see below.

Extensive monitoring

During summer 2008 we performed one extensive sampling of animals in Val di Non. This site was monitored also in 2002 and the seroprevalence for LCMV was 13.8% (9/65). In order to isolate the virus, we needed to collect more organ samples for further analyses. Animals were collected with live traps (Ugglan) and then euthanized back in the laboratory with Isofluorane.

SMI - Sweden

Rodent sampling at NILS sites (national biodiversity monitoring sites) were continued. We also simultaneously sample surface water for *Francisella tularensis holarctica* screening, and other water borne pathogens.

Monitoring viral loads in rodents (IMI – Slovenia)

In 2008, biannual rodent trappings were conducted on four locations in June and September. Even if the rodents are chronically infected by hantaviruses, the shedding of virus is relatively short term (see above). Hantavirus RNA was demonstrated in all tested internal organs and blood samples of 14 naturally infected rodent hosts. However, the concentration of a specific virus differs with regard to the virus / host and to the organ tested. DOBV, the most virulent of these viruses, showed considerably higher viral loads in all internal organs and blood samples of infected *A. flavicollis* hosts, as did PUUV and SAAV in their hosts. In addition, high RNA concentrations of DOBV (100 - 10^8 copies/ml) and PUUV (10 – 100 copies/ml) were demonstrated in rodents' blood samples despite the presence of IgG antibodies. Virus concentrations in urine varied from 10 to 10^6 copies/ml, with a mean value of 5,85 x 10^3 copies/ml. Compared with the viral loads in other internal organs, the detected concentration is lower than expected. Also it seems that the viral load in the organs declines in the course of infection (fig. ROBO-12). Knowledge on shedding and viral loads helps to plan the risk control and to understand the timing of the greatest risk.

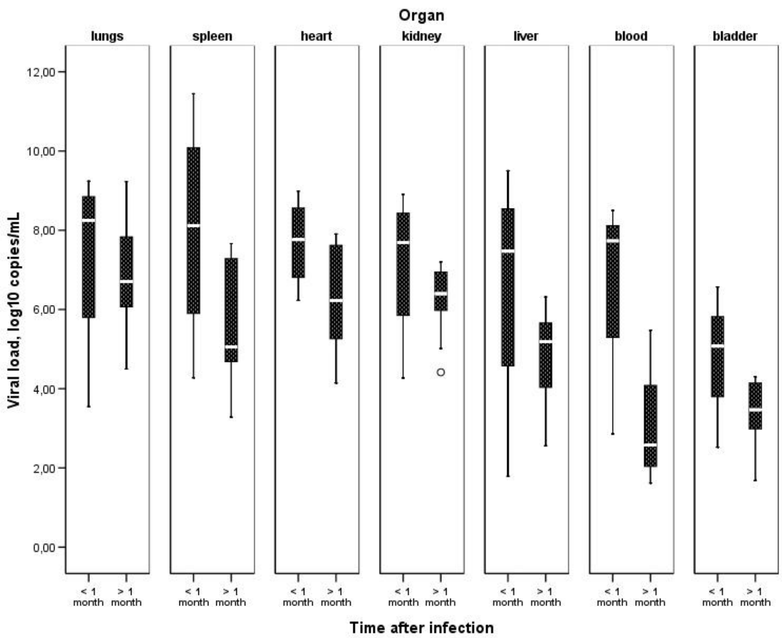


Fig. ROBO12: Hantaviral load in tested internal organs with respect to the time of infection (< 1 month, recent infection; >1 month, old infection)

The genetic variation (dynamics of reassortments in PUUV in cyclic host populations will be studied in Finland (UH-HI, METLA).

Achieved results: The reassortment genetics have been studies in detail in two study areas in Finland, and the commonness of reassortement events in rather small local scale has surprised us (Razzatuti et al 2008). In western Lapland, we have found the contact zone between northern Scandinavian PUUV lineage and the eastern lineage. This contact zone is no way congruent with those of host lineages; host contact zones are at least 500 km away. At this site, within the one host lineage, all kinds of PUUV reassortments have been found, from pure N Scandinavian to pure Eastern, and all combinations between them. This study site at Pallasjöärvi will be a gold mine for reassortment studies. Our studies suggest that there is selection among reassortments and some of the forms are viable. Reassortments at contact zones have been considered more virulent than parental strains. We are also analyzing a recent fatal human case in Central Finland to see if the death was caused by a possible reassortment strain (rodent samples collected around the house, samples from the victim available), or if the victim was HLA B7 homozygote that predisposes to severe NE.

In addition, SMI has conducted analyses of field samples to more precisely determine the contact zone of bank voles and PUUV in northcentral Sweden, i.e. the border/contact zone between South-Scandinavian vs. North-Scandinavian lineages, resulting from the postglacial re-colonization of the Scandinavian Peninsula.

Studies on the role of MHC diversity in host fluctuations and rodent host risk will continue (GBGP).

Achieved results: The independent recent studies stress a higher hantavirus transmission between relatives in rodents. This was documented by our team for the bank vole and the common vole in Jura, Eastern France. Rodent behaviour like winter kin-clustering, shared colonies within matrilines, delayed dispersal during high densities, and/or genetic effects on the susceptibility could explain this pattern. These studies were conducted in geographic region of relatively low prevalence in hantavirus in their rodent reservoir (about 10% for both voles in France). We tried to confirm the role of relatedness as general features for hantavirus transmission among rodents from a geographic region with a high PUUV prevalence with K. Tersago and H. Leirs. Three hundred and fifty four bank voles serotyped for *Puumala*, were genotyped for 7 microsatellite loci. *Puumala* prevalence in voles varied between 22 and 68% depending on sampling dates and localities. Contrary to previous works, no systematic statistical linkage was found between rodent relatedness and hantavirus transmission. Over 10 samples (each corresponding to a given locality at a given sampling date) only one showed higher relatedness between seropositive than between seronegative ones. This sample was the one with the lowest *Puumala* prevalence (22%). Although much work has to be done on the data set, in particular to rule out potential bias in our statistical approach when prevalence are high, we hypothesize that transmission between relatives may predominate in condition of low prevalence but would be overwhelmed by other transmission modes (either transmission between unrelated or *via* the environment) when prevalence are high.

Molecular variation of the bank vole, reservoir of *Puumala virus,* continue*s* with two objectives: (1) to give a full description of the evolutionary history of bank voles in Europe, and (2) describe the molecular variation at immune genes involved in the defence against *Puumala virus* at the same European spatial scale. Both objectives were pursued in order to understand why *Puumala virus* and HFRS are not distributed similarly over the whole distribution area of the reservoir. Thanks to intensive work in the past few years within the EDEN consortium, the evolutionary history of the bank vole has been precisely documented in Europe. Moreover, the description of the molecular variation in the *DQA* gene (one important immune gene involved in the control of *Puumala virus* infection) over whole Europe has been completed and a paper submitted. Data on a second immune gene, *DRB* gene, was just acquired. We have now produced an impressive amount of molecular data in the rodent reservoir to be compared with that in the *Puumala* virus. This could be one of the main objectives of the last year of the EDEN project.

The geographic distribution of the neutral genetic variability in *Myodes glareolus* has been shaped by the alternation of habitat contraction and expansion throughout the Quaternary climatic oscillations. It results in six mitochondrial lineages, which corresponds to major glacial refugia. Using single-strand conformation polymorphism analysis (CE-SSCP) and sequencing techniques, we characterized the genetic variability of the MHC class II DQA gene, known to be under selective pressure from pathogens, and in particular hantaviruses, due to its predominant role in the initiation of the immune response in mammals. 19 DQA exon 2 alleles were detected over the whole European distribution area of *M. glareolus*. These alleles come from at least two different, and probably recombining, loci. Moreover, strong signatures of positive selection were detected within the antigen binding sites. Lastly, the DQA gene did not display a clear geographical pattern contrary to what was observed for the neutral genetic variation. These results clearly support that the MHC class II DQA gene experiences balancing selection that creates and maintains its polymorphism, and that consequently erases the footprints of *M. glareolus*' phylogeographic history. Previous detection of associations between hantavirus infection and MHC polymorphism raises the question about the potential reciprocal influence of *M. glareolus* / PUUV coevolution on the distribution of MHC genetic variation at a large geographical scale. A comprehensive assessment of the phylogeographical variation of immune genes involved in hantavirus control could thus in theory help to predict spatial limits of hantavirus occurrence and emergence. Challenging this view, we did not find any association between the distribution of allelic variation in DQA exon 2, and of PUUV. A potential explanation resides in the strong genetic spatial and temporal variation in PUUV. Different MHC glycoprotein could be involved in the recognition of these different epitopes. Another likely explanation is that the distribution of DQA exon 2 genetic variation results from many-to-many gene-parasite coevolution than from one-to-one gene-parasite coevolution. A better assessment of the risks associated with PUUV emergence at a large geographical scale would be expected from the study of genes specifically involved in *M. glareolus* / PUUV interactions.

Concerning the reservoir, the study of molecular diversity of immune genes involved in the control of PPUV infection is still in progress. A synthesis of immune mechanisms involved in the control of PUUV in rodents was published in relation with similar knowledge gained in humans. Interaction between immune function and cyclic demography in rodents, and its consequences on pathogen emergence was documented in several publications. We also related positive and negative correlations between some allelic forms of 2 MHC (major histocompatibility complex) genes (*DQA* and *DRB*) and PUUV infection in bank voles. Such associations are now investigated in other parts of the distributional area of the bank vole thanks to the collaboration with Finnish and Belgian teams within the EDEN consortium. A new molecular technique has been developed in the molecular biology laboratory to gain more rapid and more precise genotyping at MHC genes in rodent populations. Lastly, molecular variation and expression in a third immune gene involved in the control of PUUV, the TNF-α, was started and gave stimulating results.

In the experiments on PUUV survival outside the host, a high inter-individual variability of *M. glareolus* in their sensibility to PUUV infection was found. All voles from these experiments were genotyped at CBGP for *DQA* and *DRB* (see above). Genetic diversity at both genes was very low, probably as a result of strong inbreeding in the laboratory-reared vole population. Due to this low variability, no association between allelic variation at both genes and the infection success was observed.

Due to their high polymorphism and frequent duplication in vertebrates, MHC genes are notoriously difficult to genotype in wild populations. Standard laboratory techniques relying on CE-SSCP analyses do not allow the distinction of every allelic form and become very difficult to interpret when individuals harbour three or more allelic forms (ie. when genes are duplicated). We developed a new approach combining 454 pyrosequencing and mark-recapture cloning, and successfully applied the method to the genotyping of voles at the highly polymorphic MHC-DRB exon 2. The method allowed accurate genotyping of 643 *M. glareolus* and 159 individuals belonging to related species. The data set is being processed and will be used to strengthen our understanding of the geographic variation of MHC-genes in relation with PUUV infection at different geographical scales.

The level of expression of the TNF-α gene is implicated in the immune response against hantviruses. A low level of expression of this gene coding for a cytokine was identified as a factor of sensibility to hantaviruses in humans. The level of expression of this gene has never been studied in wild rodent populations. We sequenced the exons 1 to 4 of the TNF-α gene along with the exons 2 to 6 of the l'Actine-ß, a housekeeping gene usually used as control in genetic expression studies. We developed specific primers in order to estimate the level of expression using the quantitative-PCR methodology. Voles trapped into two different European areas were studied for gene expression. A first sampling was constituted in France, in an area of low hantavirus prevalence (10%). The second set of animals was from a highly endemic area in Finland (Konnevesi, 40%). In both areas, seropositive and seronegative voles were studied. A total of 88 voles were analysed: 44 from France, and 44 from Finland. Triplicates for RNA quantification were systematically done. Within the high PUUV infection prevalence area, the mean expression of the TNF-α in voles was four-fold reduced compared to that in the low prevalence area. In the high prevalence area, no significant difference between infected and non-infected voles was observed. In contrast, expression in infected voles was significantly reduced, relative to non-infected ones, in the low prevalence area. These preliminary results have to be completed using another population coming from a medium prevalence area in North Eastern Germany. Further developments will be directed to the analysis of the genetic variation at the promoter of the TNF-α gene. Studies conducted on humans demonstrated that differences in the expression of the TNF-α gene can be induced by SNP (single nucleotid polymorphism) at the vicinity of the promoter of the TNF-α gene.

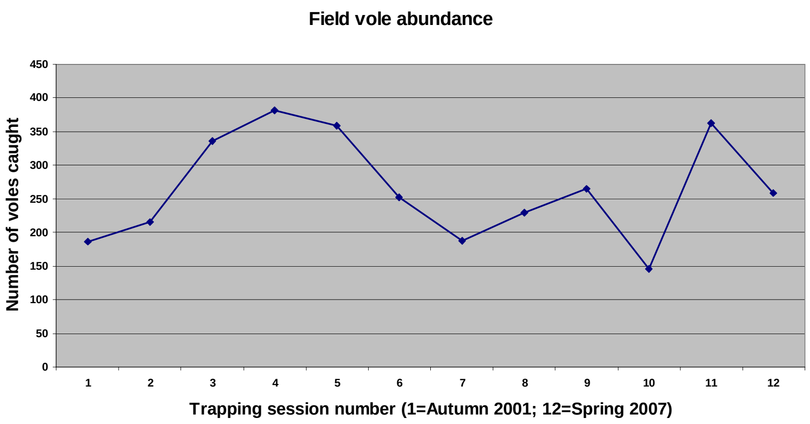
In addition, the phylogeography of *Apodemus agrarius* has been completed. The species carries Hantaan virus in the Far East but Saaremaa in western Palearctic. In this case there seems to be a congruence between viral stains and host phylogeography. Also the phylogeography of the bank vole in northern Europe and Russia has been completed.

The dynamics of cowpox in multispecies rodent host communities will be studied in more detail - are there primary and secondary hosts in reality, even if the virus seems to infect a multitude of rodent species (ULIV). Genetics of cowpox in Europe and adjacent areas will be studied (UH.HI, GBGP).

Achieved results: Cowpox (orthopox virus) genetic methods were updated (UH.HI; Putkuri et al 2009). Cowpox occurrence was screened in Belgium (UA), Estonia and Latvia (METLA). In Latvia, cowpox seemed to be mostly connected to early successional wild meadows.

Cowpox environmental studies in UK

The aim is to study the interactions between the environment and vole and infection dynamics. In close collaboration with the Louvain group, we have selected appropriate areas of Kielder Forest for further investigation. This area is a very dynamic (i.e. altering over time), man-made environment. It consists of patches of mixed and dense coniferous forest, with clear-cut areas amongst them at many different stages of development, along with different types of grassland, moors and agriculture areas.



A number of classes of vegetation were chosen to represent a wide range of types of vegetation in Kielder Forest. Thanks to this, we were able to come out with some preliminary classification of suitability of habitat for field voles. All types of vegetation eventually fell into one of three categories:

a. suitable for voles, hospitable (yellow in Fig. 13.)
b. acceptable for voles (e.g. allowing migration) (red in Fig. 13)
c. unsuitable for voles, inhospitable (green in Fig. 13)

Additionally, water bodies were added to the display to allow easier orientation.

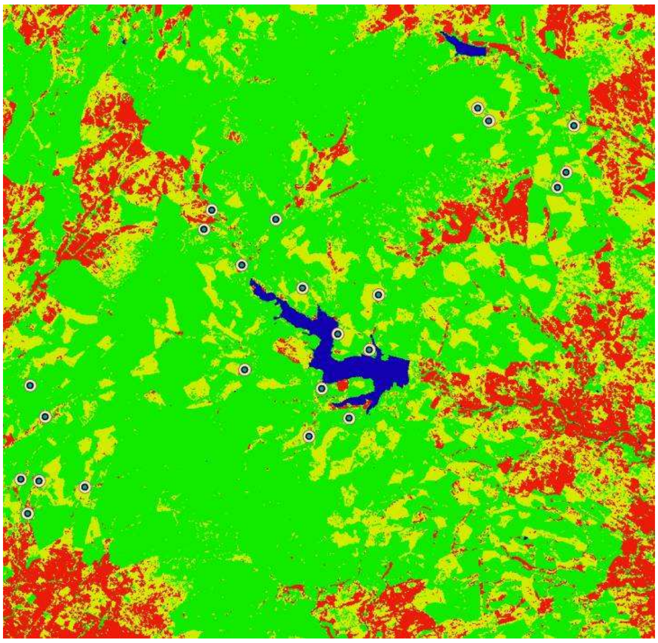
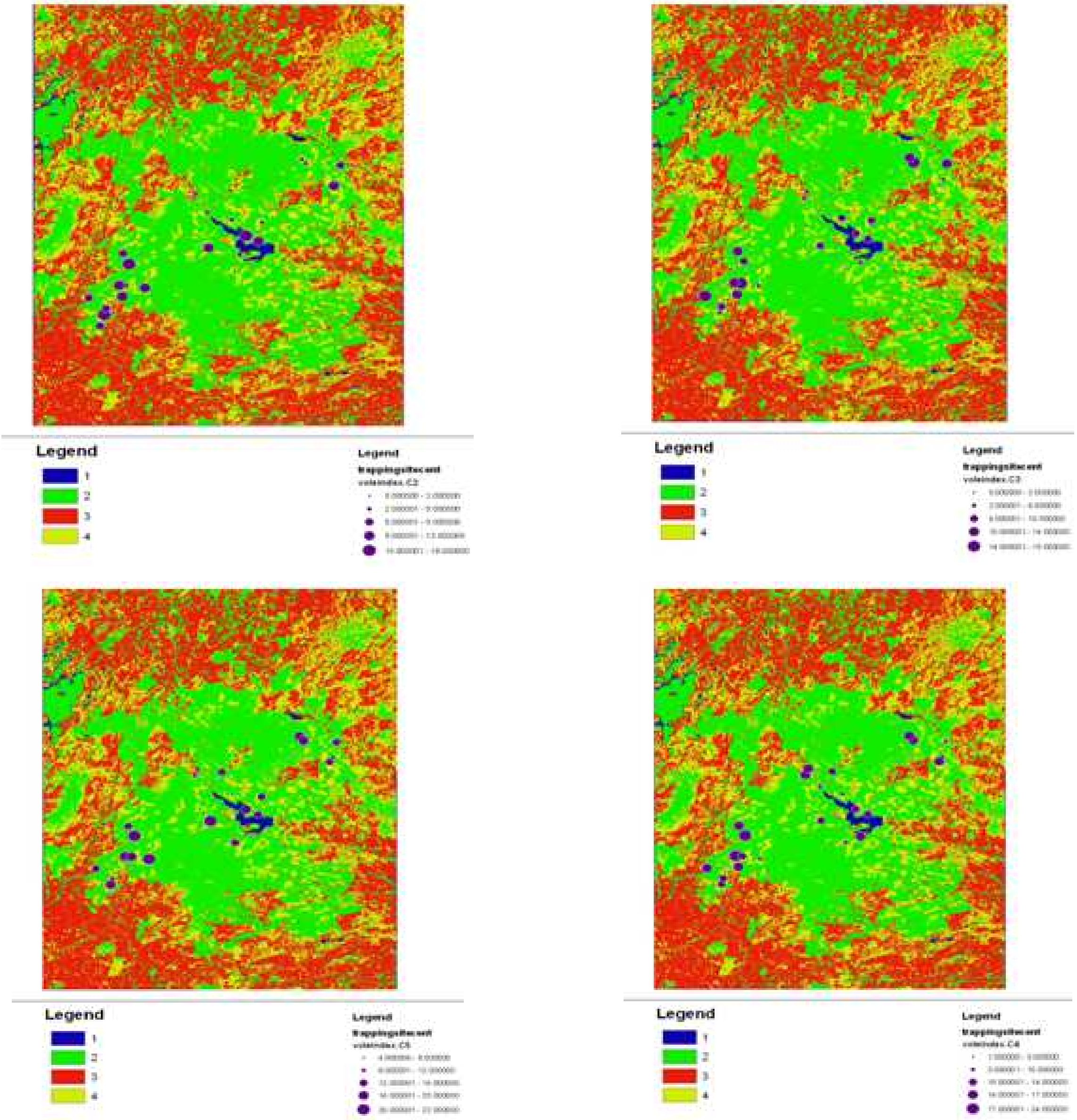


Fig. ROBO 13. Three classes of habitat (green – forest, yellow – grasslands, red – bare soil, fields, other unsuitable habitats), water – blue; dots represent the locations of trapping sites

Using NDVI, we are able to monitor the beginning of the vegetation season (rapid increase of NDVI values), how long the vegetation season lasts, how quickly it increases and decreases, the highest values it reaches and when the season ends. Productivity can be assessed as well, by combining values of NDVI from the whole vegetation season. These data can be obtained for almost every trapping site in Kielder forest and for every season, starting in 2000. This will allow us to make comparisons between seasons and trapping sites and relate these in turn to variations in vole abundance and in the prevalence of cowpox virus (and other infections).



Four out of 12 images of changing field vole abundance across time and space. Top left – spring 2001, top right – autumn 2001, bottom left – spring 2002, bottom right – autumn 2002

Detailed genetic work on LCMV-complex (arenaviruses) will be started with the help of the new Finnish grant (METLA, UH.Hi. SMI, GBGP, CEA, ULIV).

Achieved results: samples from Finland, Estonia, Latvia, Turkey, and France have been used for detailed genetic work. LCMV–type antibodies have been recently found in Slovenian rodents (IMI). Unlike hantas, horizontal and vertical transmission can lead to different outcomes with arenas: the former may cause only transient viremia while the latter may lead to chronic infection. Therefore, our strategy has been to screen for fresh, acute infections in rodent populations where seropositives have been found in case seropositives do not have viremia anymore. So-called "universal primers" developed for arenaviruses do not work for LCMV viruses. Out new data from Slovenia, Italy, Estonia, and Latvia further document our earlier findings that LCMV, or more probably, LCMV-type viruses, widely occur in many rodent species in Europe. We have developed new primers and sequencing methods, but so far we have not been able to recover genuine new sequences (and strains) except for the real LCMV from *Mus*, showing that really different strains occur in European rodents. Also, IMI failed to recover LCMV sequences in a material of 300 rodents with 17 LCMV seropositives.

As robos, the dynamics of LCMV also depend on their host dynamics. The first and only data so far on the dynamics of LCMV strain in Europe come from Trentino (CEALP). These data clearly indicate that masting affects also LCMV commonness and dynamics (Tagliapietra et al, submitted). It would be logical to assume that LCMV dynamics in humans follow this masting-induced pattern, but no data so far exist to assess the long-term patterns in humans. In fact, if there are several LCMV type strains in Europe, we do not know the infectivity of these putative strains, even if we know that LCMV-type antibodies are common in humans.

Erreur ! Des objets ne peuvent pas être créés à partir des codes de champs de mise en forme.

Fig. ROBO14 - First outdoor experiments on the survival of PUUV outside the host (UA).

Due to changes in Belgian regulations (viz. the risk classification of PUUV), planned experimental studies on extra-host survival of the Puumala virus could not be executed until now. Recently, new financial support was found for adapting the UA rodent section to the new regulations. This will enable us to start up the planned experiments in the future.

First outdoor experiments on the survival of PUUV outside the host (UA).

As a pilot study, we take the use of households as sentinels in the high endemic region to PUUV infections in northern Sweden and Finland, where inhabitants will trap rodents and take notes on in- and near house trapping success of bank voles to be related to e.g., weather (SMI, METLA).

ACHIEVED RESULTS: In autumn 2008, at three study sites in Finland, local people trapped rodents invading their houses and storage buildings. In this Finnish study, the emphasis was on *Apodemus flavicollis* and cowpox and LCMV viruses. Also other bacterial zoonoses will be analyses together with food hygienists in Helsinki University to assess if this autumnal invasion by mice is a health risk. Material has been trapped and processing has started.
As a pilot study, SMI takes the use of households as sentinels in the high endemic region to PUUV infections in northern Sweden, where inhabitants are trapping rodents and taking notes on in- and near house trapping success of bank voles to be related to e.g. weather.
Further screening of serum was achieved from wild boars (*Sus scrofa*) and techniques were developed for more rational sampling to detect antibody response to various pathogens, also those from rodents, i.e. PUUV, TBEV and *Brucella*. TBE and *Brucella* positive samples have been found.

Deliverables

D Robo-10. Further priming of highly sensitive real-time PCRs and completing the rodent-borne virus microarrays (M 36, 42). Methods reviewed for hantas and cowpox, Work in progress for LCMV, and will continue. No a great breakthrough yet on LCMV.

D Robo-13. Publications on rodent and virus distributions, genetics and phylogeographies, transmission dynamics, virus-host competence, and viral kinetics during the infection (M36) Several papers published and being published (see the list), and more is coming.

D Robo-14. GIS analysis of spatial data sets collated at both high resolution longitudinal study sites (M30).

Belgium papers published and Finnish and UK work in progress.

D Robo- 17. MHC genetic detailed.

Good progress in comparison of PUUV, Mt Dna data and immunogenetics.

D Robo-18. Research plan to study the affect of forestation of previous agricultural land in Eastern Europe on the habitat selection and distribution of rodent-borne and TBE- carrying rodents (M30). The field work in Latvia was done, and spring material was analysed. Autumn material is being processed.

D Robo -19. EDEN Robo documents on field sampling and animal ethics in wild rodent research (M36). Still in progress, steadily forward.
Milestones and expected result M ROBO-09 - Publications on all presented topics and second generation maps of observed rodent and rodent-borne viral reservoir presence in Europe allow for fine tuning of the sampling campaign(s): monitoring network and cross-sectional sampling (M36-42). In accordance.

WP ROBO 5 – Data management and cross disciplinary modelling

Work package number	Robo5	Start date or starting event:	37			
Participant id	METLA	CBGP	UA	ULIV	CEA		Total
Person-months / participant	2	3	12	4	2		23
Objectives The objective of this WP is to model and predict, using data generated by WP1-3-4, spatial and temporal transmission patterns for all major emerging hanta- and other roboviruses in Europe using an integrated health/environment approach.							

Work performed during previous reporting periods

A published model for hantavirus (Sauvage et al. 2004) has been simplified and used to find a preliminary equation for R0. The most interesting feature of Sauvage's model after "dissection" is that the virus is more sensitive to parameters determining abundance of rodents than it is to survival of the virus outside the host. Still, indirect transmission is necessary to explain the observed seroprevalences but abundance (and dynamics) of hosts is more important for determining persistence of the virus (and whether there is a real risk to humans).

Environmental parameters were related to all trapping grids in Belgium and a risk model was made on the PUUV prevalence and density of bank voles. This work was done in cooperation with the high-resolution HIT team. Finnish transmission data have been started to be modelled. The landscape variables in relation to rodent-virus spatial patterns at the Finnish intensive site started. LR analyses of Finnish human data started. The LR landscape patterns of rodent-borne viruses in the French intensive site completed.

Description of work

Much of the work described in this workpackage has been delayed due to the transfer of the UA modeller (postdoc) from UA to the matmod HIT team. This position has now again been filled and the integrated analysis work will now be conducted in detail as planned. In addition cooperation with Lyon group (Sauvage, Pontier) has now alos been established to further reinforce this activity and catch up with delayed analysis.

Mathematical modelling (chaired by UA)

A published model for hantavirus (Sauvage et al. 2004) has been simplified and used to find a preliminary equation for R_0. The model includes both the direct and indirect routes of transmission. Together with the high-resolution HIT team (UCL), this will be the basis for relating remotely-sensed landscape variables to (i) the epidemiological parameters in the R_0 expression, and (ii) directly to the serological data on hantavirus in bank voles. With such an expression we could generate maps of where the virus should be able to invade and persist, and this model includes the time of virus survival outside the host. Since there are two routes of transmission it may also be desirable to use the new framework (the quantity T1) Roberts & Heesterbeek. Our plan is to relate the modelling work as closely as possible to the high resolution imagery work. An ideal outcome would be to have an expression for R_0 that includes the landscape variables identified as important by the work with the high resolution imagery.

Achieved results: A new modeller will start.

Modelling transmission dynamics

The Belgian (2004-) and Finnish (2002-) Puumala hantavirus longitudinal transmission studies and the longitudinal monitoring by CEA in N Italy (2000 -) in very different environments are used to test the dynamic models.

Modelling of the Finnish and Belgian long-term intensive monitoring/transmission data will start - a new postdoc in UA and cooperation with Lyon group (Sauvage, Pontier).

Achieved results: Progress in Belgian analyses (see other parts of the text).

Some further discussion with METLA and Lyon group but the real work starts early next year with the completion with the longitudinal data.

Predicting hanta dynamics in Finland

We have used a large and long-term data-set (13 years, 2,317 human NE cases and 4,025 trapped bank voles) trapped in Central Finland 4 times a year to model the connections of bank vole dynamics and human monthly NE. The data indicate that the number of human infections shows both seasonal and multi-annual fluctuations and is influenced by the phase of vole cycle and time of the year and follows vole abundance with a lag of a few months. Interestingly, no clear relationship was found between PUUV dynamics in voles and human epidemics, and the possible explanations are discussed. Our results indicate that human hantavirus epidemics may be accurately predicted solely by the population dynamics of the carrier species, even without any knowledge about hantavirus dynamics in the host populations. (Kallio et al submitted EDEN 0111). However, it is known from other regions in Finland, that sometimes the human NE peak coincides with the increase phase, sometimes with the peak phase of the rodent cycle, suggesting that it highly relevant to identify the factors affecting the transmission among rodents.

Trentino LCMV data were analysed: see above (a manuscript was submitted).

Low resolution HIT

Belgium: The risk map has been combined in a GIS with the sites in Belgian with field data on rodent abundance, virus presence/absence and sometimes even virus dynamics. A more informed modelling exercise is in progress. Additional monitoring in the selected field sites was carried out during summer 2007 (new epidemic year in Belgium). These data will allow us to test the hypotheses on PUUV distribution, threshold and dilution effect, formulated earlier.

Achieved results: *Cluster analyses*

UA seeks to analyse the spatio-temporal distribution of NE cases in Belgium to identify high risk clusters through space and time. The relationship between recent changes in NE incidence in Belgium and the resulting spatio-temporal pattern are addressed. Additionally, we focus on the vole-to-human transmission mechanisms occurring before, during and after the major outbreak year 2005.

NE data were provided by the Institute of Public Health (IPH). Detailed data were available for the period 1996 until 2007. NE patients were georeferenced according to their residential municipality in the IPH database. Data on the yearly population size for each municipality were provided by the National Institute of Statistics (NIS). Spatio-temporal cluster analysis shows 3 significant high NE risk clusters in Belgium: while 2 of them are significant during the whole study period, a 3rd small cluster in eastern Belgium suddenly appears in 2003 and remains significant until 2007. This manuscript is under construction.

Finland: The Finnish 11 y data of human hanta diagnostics (more than 15 000 cases, organized with the help of National Public Health Institute) will be analyzed on the county (18 central hospital districts) basis in relation to rodent dynamics and relevant climatic variables (temperature, snow fall) and landscape variables. In addition, rodent/virus transect data 2001-2005 (two lines, 30 sites trapped in autumns across Finland; 8000 rodents) will be analysed in relation to general landscape and environmental patterns (low resolution) and human incidence patterns.

Achieved results: The large data set has been sent to Oxford for LR analyses. The transect data in being analysed. The forestry impact paper (see WP 4) is being written.

Sweden: Transect trapping started in autumn 2005, analyses from 2005-07 will be done in relation to the in general Swedish biodiversity monitoring grids with all the GIS information available. Swedish human data being analyses by together with LR HIT

Achieved results: Environmental conditions at 862 geo-referenced sites of human PUUV exposure (in 1991 to 1998) in northern Sweden were extracted from satellite data derived from the MODIS sensor on the NASA Terra satellite and temporal Fourier processed to extract environmental signatures or finger-prints of seasonality, using middle infra-red, day- and night-time Land Surface Temperatures, and the Normalised Difference and Enhanced Vegetation Indices data. The HFRS and satellite data were used within a bootstrap, non-linear discriminant analytical framework to produce risk maps of PUUV exposure.
Overall, kappa values were highly significant. Elevation of the sample sites was the best discriminator of HFRS presence and was selected in 99 of the 100 models, followed by the mean day-time Land Surface Temperatures selected in 96 of the models. These models show that in Sweden the disease is present in areas of lower temperature and at lower altitudes compared with areas of disease absence. The map was validated by e.g., observations of PUUV in bank voles at false positive locations, i.e., areas with no HFRS cases but yet predicted as high risk.
How this combination of low temperature and altitude determines successful PUUV circulation and/or bank vole numbers requires further investigation. PUUV viability outside the bank vole is known to benefit from lower temperature. Low altitudes in northern Sweden imply less snow-cover, as compared to higher altitudes. Lack of snow may drive bank voles to dispersal, which can result in the invasion of human dwellings.

Italy: Cross sectional trapping to be combined with low resolution analyses.

Achieved results: In progress.

France: Rodent dynamics and virus occurrence at different scales (see WP4) will be analysed.

Achieved results: Paper on landscape dynamics of PUUV published and cowpox patterns in rewriting.

Slovenia : One main study region (the hot spot area) will be monitored annually. Long term material (1985 -) exists for with exact source data for human cases with virus species defined.

Achieved results: Long term human data sent for LRRS analyses.

UK: LR- and HR -analyses of cowpox virus spatial dynamics in field populations in Kielder area will continue.

Achieved results: Have continued.

High resolution HIT.

Will contrast the Belgium, Finnish and N Italy patterns.

In Belgian patchy (fragmented) forest environment (and this pattern occurs widely in temperate Europe) a database of field knowledge have been collated and proposed 45 sites at which high resolution imagery should be obtained. We will characterise the landscape surrounding each of the 45 sites in two ways and then go on to fit statistical models where serological prevalence of hantavirus is the response variable. We would like to use medium-high resolution images to generate a set of explanatory variables. These variables would fall under two categories (see WP1);

(a) Connectivity. How well connected the landscape is, particularly of habitat favourable for bank voles (e.g. forest), will be important for determining the presence or absence of the virus. Local extinction is probably not uncommon.

(b) Biotope composition. This will roughly determine the abundance of voles in the landscape (the carrying capacity if you like) and this in turn is likely to have a marked effect on serological prevalence because transmission has often been thought to be density-dependent. There may even be an abundance threshold (a critical community size) operating such that the virus is consistently absent from sites where vole numbers are generally low. For this part of the work we are thinking principally to explain presence/absence rather than serological prevalence. For 9 of the 45 sites, spatially concentrated in Wallonia (within a 50 by 50km area), we have highly detailed longitudinal CMR data with which, for example, we can observe seroconversion of individual bank voles. We would like to characterise the "landscape" for these sites in a much more detailed way, i.e. matching the quality of the data on the rodents (the response variables for hantavirus here can be presence/absence overall, but also in some seasons, or even demographic differences in seropositive animals, etc.). At this scale we think of connectivity as the possibilities for bank voles to move between forest patches along hedges, creek banks etc. And we think about habitat quality as the type of forest, the type of undergrowth, the soil type, local climate (at the grid level, meaning that for example local aspects like slope, exposure etc also can be important). The aim here is to better our understanding of the mechanisms underlying serological prevalence; we can attempt to explain the variation in prevalence at the nine sites but also within grid variation. Finally we would like to look at Soil conditions/local climate. Here our reasoning is that there is some evidence that indirect transmission (virus shed in the urine persists in the environment and is subsequently transmitted to susceptible voles that inhabit neighbouring territories) is important for determining both maintenance of the virus and prevalence. It has been previously observed that serological prevalence and/or serological conversion is correlated with wet and humid terrain.

As contrast to Belgium, the forest coverage in boreal zone, e.g. in Finland is extensive, and connectivity resolution scale is not a problem for rodent and virus dispersal. Surrounding our local intensively monitored transmission study site, we have smaller "satellite" grids (i.e. replicates) within 5 x 5 km in a continuous forest environments, and we use HR analyses to study the possible differences in vole and PUUV presence, persistence or absence among these grids in the course of rodent cycles. The patchy survival of the virus in a homogenous environment during host crashes could explain the quick response of the virus to the increase of the host population in boreal zone, and would characterise the differences in viral dynamics and distribution in various parts of Europe.

Northern Italy: Intensive monitoring on several grids. HR analyses planned.

Achieved results: Finland:
Joint work between METLA and HR HIT (Lambin team) was started to integrate Finnish field data of bank voles and PUUV infections among them with HRRS data. We aim to determine environmental variables (measured both from satellite images and by surveying the habitat on trapping areas), that are linked to either bank vole or Puumala virus abundance. The final model selection is still underway, but according to preliminary analyses, bank voles favour spruce-dominated, old forests with a low photosynthetic activity. On the other hand, the virus is best sustained in mature forests, where vegetation shows great heterogeneity. In addition, the forestry impact analyses support the rather "universal" occurrence of the bank vole and PUUV in the northern landscapes, though the detailed analyses will show if there are specific survival pockets for PUUV.

Belgium
In collaboration with the High-resolution HIT team we worked on a model on PUUV infection prevalence and density in bank voles. Our hypothesis was that environmental conditions controlling the direct and indirect transmission paths differ, such that the risk of transmission to humans is not only determined by host abundance. We explored the relationship between, on one hand, environmental variables and, on the other hand, host abundance, PUUV prevalence in the host, and human cases of NE.
Linear regressions showed that landscape attributes, particularly landscape configuration, influence the abundance of hosts in broadleaf forests. Based on logistic regressions, we show that PUUV prevalence among bank voles is more linked to variables favouring the survival of the virus in the environment, and thus the indirect transmission: low winter temperatures are strongly linked to prevalence among bank voles, and high soil moisture is linked to the number of NE cases among humans. The transmission risk to humans therefore depends on the efficiency of the indirect transmission path and of course patterns of human behaviour.
One should therefore distinguish between environmental factors related to the abundance of hosts such as land-surface attributes, landscape configuration, and climate - i.e., host ecology, - and environmental factors related to PUUV prevalence, mainly winter temperature sand soil moisture – i.e., virus ecology. Beyond a threshold abundance of hosts, environmental factors favouring the indirect transmission path (soil and climate) could then better predict the number of NE cases among humans than factors influencing the abundance of hosts. Linard, C. et al. 2007. Environmental conditions and Puumala virus transmission in Belgium. International Journal of Health Geography. 2007, 6:55. (EDEN 0060).

Additionally we collaborated with high-resolution HIT-team on their spatial model on human PUUV infection. Linard C, Lamarque P, Heyman P, Ducoffre G, Luyasu V, Tersago K, Vanwambeke SO, Lambin EF., 2007.Determinants of the geographic distribution of Puumala virus and Lyme borreliosis infections in Belgium. International Journal of Health Geography. 2007, 6:15 (EDEN 0043).

Geographical modelling of the spread of hantavirus in western Europe by means of Ecological Niche Modelling (ENM)

The observation that Puumala hantavirus and human Nephropatia epidemica cases are characterized by a focal distribution in western Europe, leads to the assumption that environmental variables may play a modifying role for the spread of hantavirus here. Indeed, the goal of this modelling part is (1) to characterize the distributional areas of hantavirus and (2) to predict 'risk' areas where appropriate ecological conditions are prevailing so that a suitable habitat for hantavirus may be present. More specifically, we search for a link between the occurrence of hantavirus and the environmental conditions that are present at these locations. To this end, we use one Ecological Niche Modelling technique, named GARP (Genetic Algorithm for Rule-set Prediction. With GARP, we develop models based on non-random associations between known occurrence points for hantavirus and sets of coverages describing the ecological landscape. We do this by contrasting ecologic characteristics of presence points to those of points sampled randomly from the rest of the study region using a series of decision rules developed by GARP in an iterative process of rule selection, evaluation, testing and incorporation/rejection. Individual human disease records from Belgium, the north part of France and Slovenia were brought together in a database. For each human case, the location where the patient lives was given. The infected locations were listed and by means of geo-coding files and world gazetteers, geographical coordinates were assigned to most locations. These infected locations were used as a proxy for location where hantavirus is present in nature. Several ecological variables describing bioclimate, ecosystem, topography and land cover are brought together in a GIS database. A big part of the environmental layer data was found on the EDEN website; through some simple steps, they were ready for integration in the environmental database. Model development and validation is still under process and will be carried out in the next months.

Achieved results: Results showed that the available data were not sufficient to make a predictive model for NE risk areas in wide areas of Europe. In order to optimize the model more data (occurrence points and environmental data) would be needed.

Deliverables
D Robo-15. Exploratory area-wide GIS model for the testing of rodent-borne disease risk assessment approaches (M42). Aiming at.
D Robo-16. Exploratory local high resolution GIS model for each field site for the testing of spatial epidemiology hypothesis and health-environment relationships (M42). In good progress.
Milestones and expected result
M ROBO-10 - Relevant spatial data are made available to include in GIS model (M 36-42). Achieved

Workpackages LEI 1 to LEI 5: Leishmaniasis

Deliverable review

Work package	Deliverable number	Deliverable description	Programmed delivery month	CNM 9 Spain	ISS 12 Italy	IHMT 15 Portugal	NHM 34 UK	EUMS 35 Turkay	LSHTM 36 UK	SZIE 37 Hungary	UB 38 Spain	UM1 39 France	UoC 40 Greece
WP1	D LEI01	Standardized spatial GIS data base of environments and climate descriptors in each study region	15	F	N/A	N/A	F	F	F	N/A	N/A	N/A	N/A
WP1	D LEI02	Standardized description and maps of environments and climate in each study region	18	F	D4	N/A	F	F	F	N/A	D4	N/A	N/A
WP2	D LEI03	Standardized spatial data base of sandfly species in each study region	15	F	E	E	F	F	N/A	[F]	D4	N/A	D4
WP2	D LEI04	Standardized maps of distributions of sandfly species in each study region	18	F	D4	E	D4	F	N/A	N/A	D4	N/A	D4
WP2	D LEI05	Report on molecular findings for sandflies	18	N/A	N/A	N/A	F	N/A	N/A	N/A	N/A	N/A	N/A
WP3	D LEI06	Standardized spatial and temporal databases of *Leishmania* species isolated from patients	15	E	F	E	C4	E	C4	F	E	C4	E
WP3	D LEI07	Descriptive analysis of spatial & temporal changes of prevalence rates of human leishmaniasis	18	E	F	E	C4	D4	C4	N/A	D4	C4	D4
WP3	D LEI08	Appraisal of an European initiative to standardize national surveillance systems and networks for human leishmaniasis	18	D4	D4	D4	D4	D4	D4	N/A	D4	D4	D4
WP4	D LEI09	Standardized spatial and temporal databases of *L. infantum* strains isolated from dogs	15	E	F	E	N/A	F	F	[F]	E	C4	E
WP4	D LEI10	Descriptive analysis of spatial changes of prevalence rates of canine leishmaniasis	18	E	E	E	F	D4	F	N/A	D4	C4	D4
WP4	D LEI11	Appraisal of an European initiative to standardize national surveillance systems and networks for canine leishmaniasis	18	D4	D4	D4	D4	D4	D4	N/A	D4	D4	D4
WP5	D LEI12	First preliminary model for 3 European regions of spatial and temporal associations between leishmaniasis prevalence, its vectors, and changes	18	N/A	C4	N/A	F	N/A	F	N/A	C4	N/A	N/A
WP5	D LEI13	Standardized spatial GIS data base of environmental and climate descriptors for all study regions (raw data sets – M24, processed data-	24	F	F	F	F	F	F	D4	D4	N/A	D4
WP5	D LEI14	Standardized descriptions and maps (GIS models) of environments and climates in all study regions (M30)	30	F	F	F	F	F	F	F	D4	N/A	D4
WP5	D LEI15	Standardized spatial data base of new records of sandfly species in each study region (M30)	30	F	D4	N/A	F	F	N/A	D4	D4	N/A	C1
WP5	D LEI16	Preliminary analysis of spatial & temporal changes of prevalence rates of human leishmaniasis in some regions in the past 20 years (M30)	30	F	D4	D4	F	F	F	C1	C1	C4	C1
WP5	D LEI17	Preliminary analysis of spatial & temporal changes of incidence rates of canine leishmaniasis in some regions in the past 20 years (M30)	30	C4	C4	C4	C1	C4	C1	C1	C1	C1	C1
WP5	D LEI18	Report on new prospective studies of canine leishmaniasis in southern France, northern Spain and Italy (M30)	30	F	D4	N/A	N/A	F	N/A	E	E	E	D4
WP5	D LEI19	The first preliminary model for all regions of the spatial association between leishmaniasis prevalence, its vectors, and climate &	30	C4	C4	N/A	C1	C4	C1	C1	C1	C1	C1

Action		Problems	
A	Preparatory phase	0	Finding sources
B	Sources identified, requests submitted	1	Ongoing data (paper archived) input
C	In progress	2	Few/incomplete data/only monthly or annual means
D	Data collected, not sent to NHM/ LSHTM/ Montpellier	3	Delay in data delivery from archive
E	Data files sent to NHM/ LSHTM/ Montpellier	4	Ongoing file cleaning, revision or seeking for missing
(E)	Text descriptions sent, data do not exist	NA	Not applicable
F	Well established techniques in progress, but [F] not followed		

Some changes: Most partners focused on obtaining new spatial distribution data for sandflies (WP2) and canine leishmaniasis (WP4), rather than publishing incomplete data. PI of partner LSHTM 36 has been ill, but a research assistant was finally recruited in September 2008 and there has been substantial late progress with analysis

Table LEI-O1: Deliverable status per LEI partner

Executive summary

Good progress was made with completing field surveys of environments (WP1), the sandfly vectors of Leishmania infantum (WP2), and canine leishmaniasis (WP4) by the LEI partners: CNM 9 (central Spain), UB 38 (northeastern and southern Spain), UM1 39 and NHM 34 (France), ISS 12 (northern and central Italy), SZIE 37 (southern Hungary), UoC 40 (Attica, Greece) and EUMS 35 (western Turkey). Concerning planning and data analysis (WP5), sub-project workshops were held at the EDEN AGM in Brno (month 39) and in Granada (month 44). Collaborations with the HITS were continued, with the aim of producing Ro biological models (with the Mathematical Modelling, the HR Environmental Change and Low Resolution modelling HITs). Data analysis and modelling were delayed by illness affecting some partners and the difficulty of recruiting a replacement researcher.

Publications

The following PhD students are currently conducting research using EDEN data:

Miss Rosa GALVEZ, CNM 9, Environmental distribution of leishmaniasis and its sandfly vectors in Madrid province, Spain.

Miss Shazia MAHAMDALLIE, NHM 34, Effects of environmental bottlenecking on the allele diversity of salivary peptides of *Phlebotomus ariasi*.

Mr Balázs TÁNCZOS, SZIE 37, Environmental distribution of sandflies in Hungary.

Miss Cristina BALLART, UB 38, Environmental distribution of leishmaniasis and its sandfly vectors in Catalonia, Spain.

Mr Sergio BARON, UB 38, Environmental distribution of leishmaniasis and its sandfly vectors in Granada province, Spain.

Mr Ippokratis MESSARITAKIS, UoC 40, Environmental distribution of leishmaniasis in Attica, Greece. [Status as EDEN student in doubt, because no publications planned.]

Publications of EDEN-LEI (with an EDEN number)

NHM 34

READY, P.D., 2008. *Leishmaniasis emergence and climate change*. In: Climate change: impact on the epidemiology and control of animal diseases (Ed., S. de la Rocque). *Rev.sci.tech.Off.int.Epiz.* **27:** 399-412. EDEN 0086.

SZIE 37

FARKAS, R. & TÁNCZOS, B., in press. *A kutya leishmaniosisa és jelentősége Európában. Irodalmi áttekintés* [Canine leishmaniosis and its importance in Europe. Literature review]. *Hungarian Veterinary Journal*. EDEN 0121.

UM1 39

DEREURE, J., VANWAMBEKE S.O., MALÉ P., MARTINEZ S., PRATLONG F., BALARD Y. & DEDET J.P., in press. The potential effects of global warming on changes in canine leishmaniasis in a focus outside the classical area of the disease in southern France. *Vector Borne and Zoonotic Diseases.* EDEN 0123.

Publications of EDEN-LEI (EDEN number not applied for)

ISS 12

SCHÖNIAN G., MAURICIO I., GRAMICCIA M., CANAVATE C., BOELAERT M. & DUJARDIN J.C. (2008). Leishmaniases in the Mediterranean in the era of molecular epidemiology. *Trends in Parasitology,* 24: 135-142.

Meetings where EDEN-LEI results and ideas have been presented

CNM 9

GÁLVEZ, R. (2008). *Leishmaniasis en la región central de España: fluctuaciones en la seroprevalencia del reservorio y la distribución de sus vectores. VI Congress of the Spanish Society of Tropical Medicine and International Health (SEMTSI)*, Segovia, Spain, 5-7 March 2008.

ISS 12

BONGIORNO G., SCORTICHINI M.G., GRADONI L., GRAMICCIA M. & MAROLI M. (2008). Environmental and climatological factors as determinants of the distribution of two *Leishmania* vectors, *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*, in the Apennine mountains of central Italy. *Proceedings of the XXV Congr. Soc. It. Parassitologia*, Pisa (PI) 18-21/06/2008, *Parassitologia,* 50 (1-2 suppl.): 100.

GRAMICCIA M., BONGIORNO G., DI MUCCIO T., GRADONI L. & MAROLI M. (2008). The spreading of Leishmaniasis in Italy: Entomological surveys in Northern and Central-Western regions. *Proceedings of the 6th International Symposium on Phlebotomine Sandflies* (*ISOPS 6),* Lima (Peru), 27-31/10/2008, P-63.

MORTARINO M., MAROLI M., BECCATI M., BUTTINONI D., CALZOLARI M.L., MERONI M., ZAPPELLINI G., BAZZOCCHI C., GENCHI C. & GRADONI L. (2008). Detection of new canine leishmaniasis foci in Northern Italy. *Proceedings of the XXV Congr. Soc. It. Parassitologia*, Pisa (PI), Italy, 18-21/06/2008, *Parassitologia*, 50 (1-2 Suppl): 160.

NHM 34

READY, P.D. (2008). *Risk assessment results for phlebotomine-borne diseases*. In: Magnitude and importance of vector-borne diseases in Europe. ECDC meeting, Stockholm, 11-12 June 2008.

EUMS 35

ERTABAKLAR, H., I CÜNEYT BALCIOGLU, I., Nermin Sakru[3], OZENSOY TOZ, S. & OZBEL, Y. (2008). First epidemiological and entomological study on canine leishmaniasis in Kırklareli Province located in Thrace region of Turkey. *Proceedings of the 6th International Symposium on Phlebotomine Sandflies* (*ISOPS 6),* Lima (Peru), 27-31/10/2008.

OZBEL, Y., CÜNEYT BALCIOGLU, I., ÖLGEN, K., SIMSEK, F., OZENSOY TOZ, S., ERTABAKLAR, H., DEMIR, S. & ZIYA ALKAN, M. (2008). Mapping of risk areas for visceral and cutaneous leishmaniasis related with distribution of vector species in western part of turkey using geographic information systems. *Proceedings of the 6th International Symposium on Phlebotomine Sandflies* (*ISOPS 6),* Lima (Peru), 27-31/10/2008.

WP LEI 1 – Landscapes, biotopes and habitats

Work package number	LEI WP1	Start date or starting event:		37		
Participant id	ISS	NHM	LSH&TM	UB	UM1	CNM	IHMT
Person-months /participant	1	1	1	1	0.5	1	0.5
Participant id	EUMS	SZIE	UoC				Total
Person-months /participant	1	0.5	0.5				8

Objectives

The general objective is to quantify the relative roles of specific environmental and climatic factors as determinants of leishmaniasis foci.

The specific objective will be to match the results with vector/ disease data from other WPs, to identify indicators of foci within the 3 initial study regions [in Catalonian Spain (Andorra-Lleida-Barcelona; UB, NHM, LSH&TM), neighbouring France (Andorra-Toulouse-Carcassonne; UM1, NHM, LSH&TM) and northwest Italy (Torino-Genova-France; ISS)] and to test predictions of foci in the countries of all partners.

Work performed during previous reporting periods

Field work was started in summer 2005 in each of the three designated regions: Catalonian Spain (Andorra-Lleida-Barcelona), neighbouring France (Andorra-Toulouse-Carcassonne) and northwest Italy (Torino-Genova-France). In a first step, Southern France was selected as a model focus, where environmental descriptors were included in a Palm database tested in the field. RS data and ground records for climate and land cover were obtained for the same region. In 2006, such work was completed in the Catalonian and Italian regions, for which RS data and ground records for climate and land cover are matched with new sandfly records in a preliminary analysis. Based on this, RS data for climate and land cover are being selected for the three eastern regions in Greece, Hungary and Turkey. The collection of ground records was extended to Turkey.

In 2007, RS datalayers were collected for the Attica focus in Greece, but not for Hungary because of limited distribution of vectors and absence of leishmaniasis, and not for Turkey because of the need to establish altitudinal transects with sufficiently high densities of sandflies and prevalences of leishmaniasis. The plan was to focus on analysis, but this was changed because of 1) the need to obtain more complete data sets for WP2 and WP4 from all regions, and 2) the illness of the PI of LSHTM 36. Nevertheless, a training workshop for the manipulation of data layers in ArcView was held in Barcelona (month 30), high resolution analysis of data from southwest France was carried out in Leuvan in collaboration between NHM 34 and the Environmental Change HIT (month 30), and priorities for collecting new field data were agreed with all partners in a workshop in Piacenza, Italy (month 31) before the transmission season in summer 2007.

Description of work

In M37-54, the emphasis will be on data processing and analysis. This will be co-ordinated by NHM (with one PhD) and LSH&TM (with one part-time post-doctoral geographer).

There will be at least one workshop to help partners analyse their data and prepare results for publication, with the date and location to be decided at the EDEN AGM 2008 (Provisionally month 42 in Granada, Spain).

Achieved results: The long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated. It was only in September 2008 that approval to recruit a research assistant to help LSHTM 36 was granted and a suitable assistant identified. A workshop was held in Granada, Spain (month 44), attended by all partners except UM1, from which it was clear that partners CNM 9, ISS 12 and UB 38 did not have adequate support from local geographers. There are now plans to assist these teams (see 1.4.1.3. above; CNM 9 was assisted by a visit from LSHTM 36 and NHM 34 in December 2008). Field collection of environmental data was continued by partners CNM 9, ISS 12, NHM 34, EUMS 35 and UB 38.

Deliverables

Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project.

D LEI13 – Standardized spatial GIS data base of environmental and climate descriptors for all study regions (raw data sets – M24, processed data-layers – M30). All outstanding raw data sets will be obtained and processed (M36). Level of achievement: completed by LSHTM 36 as each partner provides WP4 results.

D LEI14 – Standardized descriptions and maps (GIS models) of environments and climates in all study regions (M42). Level of achievement: completed by all designated partners, but data of ISS 12 and UB 38 needs to be reviewed by LSHTM 36 and NHM 34.

Milestones and expected results

M LEI05 - Preliminary spatial model outputs are available and allow for 1) spatial analysis of vector/ disease/ environment associations in first 3 study regions (WP5; M18), and 2) extrapolation to other regions (M36). Level of achievement: completed for Madrid, France, Turkey and Greece, but a timetable for completion is needed for ISS 12 and UB 38.

M LEI26 – Preliminary spatial model outputs are available and allow for extrapolation to other regions. Planning workshop in Granada, Spain, for all teams (M42). Level of achievement: workshop completed in June 2008 (M44).

WP LEI 2 – Vector bionomics and competence

Work package number	LEI WP2	Start date or starting event:				37
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	2	5	0	16	5	1	5
Participant id	UB	UM1	UoC				Total
Person-months / participant	5	1	5				45

Objectives

The general objective of this WP is to explore the variations and recent changes in the abundance of vector species and races in contrasted ecotopes.

The specific objectives are:

To analyse the geo-referenced past records of the sandfly vectors of leishmaniasis.

The 5 most experienced teams will continue to re-sample some areas in the third leishmaniasis transmission season (May-October 2007), in their study regions in Catalonian Spain (UB 38), neighbouring France (NHM 34, LSH&TM 36, UM1 39) and northwest Italy (ISS 12) (Details in WP1). In the summer of 2007, eastern Mediterranean teams will continue sampling in their regions. These new collections will transect the range of bioclimate zones and altitudes in each region, inside and outside previously studied leishmaniasis foci. The results will be matched with environmental and disease data from other WPs, to identify indicators of foci.

To investigate population structure and dispersal in relation to environmental variations using molecular characterization of some sandfly species in some regions.

Work performed during previous reporting periods

An historical database on absence / presence and density data for each sandfly species, within foci and non-foci, based on past records was developed and completed by all partners. New collections specifically targeted the range of climate and environmental zones in each of the study regions, starting with Catalonian Spain, France and Italy, then Spain (Granada, Madrid), Greece (Athens), Hungary (southwest) and Turkey (Centre-South). Population structure and dispersal in relation to environmental variations were investigated using molecular characterization of some sandfly species in some regions.

In 2006-7, the historical database was updated, and the database for new collections was used by CNM 9, ISS 12, NHM 34, EUMS 35 and UB 38. Data processing and analysis was carried out for the data from the model region of southwest France (NHM 34, LSHTM 36), with the priority for the other regions being changed from analysis to the collection of new data to obtain more complete data sets. Training for other teams was provided in two workshops, attended by all the designated partners. A workshop in Barcelona (M30) focused on further training of the Spanish teams to use ArcView software with a statistical package (STATA) for spatial analyses. In a workshop in Piacenza (M31), the focus was on planning sandfly (and canine leishmaniasis) surveys. New spatial (altitudinal) and/or temporal (seasonal) sandfly surveys were carried out in July-September 2007 by partners CNM 9 (central Spain), ISS 12 (in Abruzzi region, Italy), NHM 34 (in southwest France), EUMS 35 (Turkey), SZIE 37 (Hungary) and UB 38 (northeastern and southern Spain). Molecular characterization of some sandfly species continued. Populations of sandflies from a range of habitat fragments in southwest France were characterized at mitochondrial and nuclear loci, to investigate the effect of habitat fragmentation on the dispersal of sandfly vectors of leishmaniasis (NHM 34).

Description of work

In the next 18 month period (M37-54), the emphasis will be on data processing and analysis. This will be co-ordinated by NHM (with one PhD) and LSH&TM (with one part-time post-doctoral geographer).

Achieved results: The long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated. It was only in September 2008 that approval to recruit a research assistant to help LSHTM 36 was granted and a suitable assistant identified. The historical databases for northern and southern Spain (UB 40) and the updated historical database for Greece (UoC 40) have not been received by the co-ordinator (NHM 34).

There will be at least one workshop to help partners analyse their data and prepare results for publication, with the date and location to be decided at the EDEN AGM 2008 (Provisionally month 42 in Granada, Spain).

Achieved results: A workshop was held in Granada, Spain (month 44), attended by all partners except UM1, from which it was clear that partners CNM 9, ISS 12 and UB 38 did not have adequate support from local geographers. There are now plans to assist these teams (see 1.4.1.3. above; CNM 9 was assisted by a visit from LSHTM 36 and NHM 34 in December 2008).

The need for new surveys to complete datasets will be assessed at EDEN AGM 2008 (M39).

Achieved results: Field surveys of sandflies were continued by partners CNM 9, ISS 12, NHM 34, EUMS 35, SZIE 37 and UB 38. However, surveys carried out in Hungary were not standardized, and this should be rectified in the last period of the project.

Deliverables

Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project.

D LEI15 – Standardized spatial data base of new records of sandfly species in each study region (M60). Level of achievement: completed by all designated partners, but data of ISS 12 and UB 38 needs to be reviewed by LSHTM 36 and NHM 34.

Milestones and expected result

M LEI08 - Spatial data layers used to generate spatial analysis models, focusing on western regions. Milestone to be discussed at AGM 2008 (M39). Level of achievement: discussed at AGM (absence of Spanish partners) and in Granada, Spain (M44), but delay in recruiting research assistant for LSHTM 36 has restricted progress.

M LEI26 - Preliminary spatial model outputs are available and allow for extrapolation to other regions. Planning workshop in Granada, Spain, for all teams (M42). Level of achievement: workshop completed in June 2008 (M44).

WP LEI 3 – Public health and human activities

Work package number	LEI WP3	Start date or starting event:		37			
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	1	1	0	0	1	0.5	0
Participant id	UB	UM1	UoC				Total
Person-months / participant	1	1	1				6.5

Objectives

The general objective is to investigate recent spatial and temporal changes in human disease prevalence.

This will also be a specific objective, now that records of past and new cases of human leishmaniasis have been standardized. A report will be prepared on how to improve both case detection, by encouraging the use of standardized molecular tools, and case reporting, by proposing a standardized surveillance questionnaire for all countries.

Work performed during previous reporting periods

From national records, databases were prepared on the spatial and temporal distributions of prevalence rates of all past and new autochthonous cases caused by *Leishmania infantum* or *Le. tropica* strains in each partner's study region. The descriptive analysis of spatial and temporal changes of prevalence rates in all 9 regions in the past 20 years was started.

In 2006-7, separate reports were prepared or updated with new cases for central Spain (CNM 9), south and east Spain (UB 38), France (UM1) and Italy (ISS 12), in preparation for a review of the epidemiological situation in southwest Europe. As in previous years, molecular characterization of Leishmania species and strains isolated from patients (classed by sex and age etc) was on-going. At the EDEN AGM in 2007 and in a subsequent workshop, partners briefly discussed how to improve both case detection, by encouraging the use of standardized molecular tools, and case reporting, by proposing a standardized surveillance questionnaire for all countries. The discussion was led by partners associated with the national reference centres for France (UM1), Italy (ISS) and Spain (CNM), but there were not enough resources to produce deliverables.

Description of work

There was little activity in this workpackage in the third 12 months, because the lack of funds required postponing some activities. This will be remedied in the next 18-month period (M37-54). The descriptive analysis of spatial & temporal changes of prevalence rates in the past 20 years was completed separately for southwest European countries, and this will be extended to all regions after a new deadline has been agreed at EDEN AGM 2008 (M39).

Achieved results: In 2006-8, separate reports were prepared or updated with new cases for central Spain (CNM 9), south and east Spain (UB 38), France (UM1) and Italy (ISS 12), in preparation for a review of the epidemiological situation in southwest Europe. As in previous years, molecular characterization of *Leishmania* species and strains isolated from patients (classed by sex and age etc) was on-going.

A report will be started on how to standardize national surveillance systems and networks, after a new deadline has been agreed at EDEN AGM 2008 (M39).

Achieved results: It was agreed to complete this objective at EMOP X in Paris, August 2008, but too few partners attended. (The new deadline is for the WorldLeish4 Congress in India, February 2009, which will be attended by key partners: UM1 39, ISS 12, NHM 34 and LeishRisk EU FP6 project).

Finally, a start will be made on a statistical analysis of spatial & temporal changes of prevalence rates in the past 20 years, after a new deadline has been agreed at EDEN AGM 2008 (M39).

Achieved results: Not achieved because of lack of time. New deadline to be agreed at the WorldLeish4 Congress in India, February 2009, which will be attended by key partners: UM1 39, ISS 12, NHM 34 and LeishRisk project.

Deliverables

Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project.

D LEI07 – Descriptive analysis of spatial & temporal changes of prevalence rates in all 9 regions in the past 20 years (M36). Level of achievement: incomplete because of knock-on effect of lack of resources in period 3.

D LEI08 – Report on how to standardize national surveillance systems and networks (M42). Level of achievement: postponed because of knock-on effect of lack of resources in period 3.

D LEI16 – Analysis of spatial & temporal changes of prevalence rates in some regions in the past 20 years (M42). Level of achievement: incomplete because of knock-on effect of lack of resources in period 3.

Milestones and expected result

M LEI27 - New timetables for completing the 3 deliverables were [to be] agreed at AGM 2008: Progress to be reviewed at the SP meeting in Granada [M42], to ensure delivery by November 2007 (M37). Level of achievement: new timetables further amended, because of lack of time and absentees from EMOP X in Paris, August 2008.

M LEI28 - Progress on completing the 3 deliverables will be monitored by SP co-ordinator at the training workshop in Granada (M42). Level of achievement: monitored, but had to be postponed because of lack of time and absentees from EMOP X in Paris, August 2008.

WP LEI 4 – Animal reservoirs

Person-month inputs are approximate because the PMR of an institute mixes P-Ms from different subprojects.

Work package number	LEI WP4	Start date or starting event:			37		
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	0.5	0.5	0	0	2	2	0.5
Participant id	UB	UM1	UoC				Total
Person-months / participant	0.5	1	1				8

Objectives

The general objective is to quantify the role of dogs (as reservoirs) in the dynamics of the disease.

The specific objective is to use the geo-referenced databases to analyse spatial and temporal changes in disease prevalence and/or incidence in relation to climate and environmental descriptors in all study regions (see WP1).

For later stages of the project, case detection and reporting need to be improved, respectively, by encouraging the use of standardized molecular tools and a standardized surveillance questionnaire in all countries.

Work performed during previous reporting periods

Previously, regional databases were prepared from national, veterinarians' and partners' databases and merged in London (LSHTM 36), from which spatial and temporal distributions of prevalence and incidence rates were plotted for all past and new autochthonous cases caused by Le. infantum strains in some regions. Prospective serological surveys were carried out by many of the teams. Isolates were identified according to guidelines defined by the sub-project.

In 2006-7, the canine leishmaniasis database was updated, and the prevalence records from Spain, Portugal and France were mapped and used to produce the first risk map for the whole of Europe, led by LSHTM 36. Additional prospective surveys, both serological and based on questionnaires posted to veterinarian clinics, were carried out by partners CMN 9 (Spain), UM1 (France), ISS 12 (Italy), EUMS 35 (Turkey) and SZIE 37 (Hungary). Isolates provided new geographical records for the sub-Alpine region of Italy (ISS 12) and Spain (UB 38). Lack of funds postponed the formulation of recommendations to standardize the national systems for surveillance of canine leishmaniasis and the method of molecular characterization of Leishmania.

Description of work

In M37-54, disease prevalence surveys will be completed in France, Spain, Italy, Hungary and Turkey, based on questionnaires to veterinarians clinics, and focusing on follow-up mailings and telephone calls.

Achieved results: These objectives were met for Greece as well as the designated partners, except for northern Spain (UB 38). However, surveys carried out in Hungary were not standardized, and this should be rectified in the last period of the project.

As for the other workpackages, the emphasis will be on data processing and analysis. This will be co-ordinated by NHM (with one PhD) and LSH&TM (with one part-time post-doctoral geographer).

Achieved results: The long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated. It was only in September 2008 that approval to recruit a research assistant to help LSHTM 36 was granted and a suitable assistant identified. Good progress has since been made.

There will be at least one workshop to help partners analyse their data and prepare results for publication, with the date and location to be decided at the EDEN AGM 2008 (Provisionally month 42 in Granada, Spain).

Achieved results: A workshop was held in Granada, Spain (month 44), attended by all partners except UM1, from which it was clear that partners CNM 9, ISS 12 and UB 38 did not have adequate support from local geographers. There are now plans to assist these teams (see 1.4.1.3. above; CNM 9 was assisted by a visit from LSHTM 36 and NHM 34 in December 2008).

Deliverables
Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project. D LEI10 – Descriptive analysis of spatial changes of prevalence rates in all 9 study regions in the past 20 years (M42). Level of achievement: This was restarted in October 2008. D LEI11 – To try to standardize the national systems for surveillance of canine leishmaniasis and the method of molecular characterization of *Leishmania* (M42). Level of achievement: postponed because of knock-on effect of lack of resources in period 3. D LEI18 – Report on new prospective studies (serological surveys and vet questionnaires) in Spain (CNM, UB), France (UM1), Italy (ISS), Greece (Uoc), Hungary (SZIE) and Turkey (EUMS) (M36). Level of achievement: completed in Granada (M44).
Milestones and expected result
M LEI17 - Preliminary spatial model outputs are available and allow for 1) first spatial analysis of disease / vector / environment associations in 3 detailed study regions (see WP1), and 2) extrapolation to other regions at a later stage (M36). Level of achievement: 2) This was restarted in October 2008. M LEI29 - New timetables for completing deliverables to be agreed at AGM 2008 (M39). Level of achievement. Discussed at AGM (absence of Spanish partners) and in Granada, Spain (M44), but delay in recruiting research assistant for LSHTM 36 has restricted progress. M LEI30 - Progress on completing deliverables will be monitored by SP co-ordinator at the planning workshop (see WP1) (M42). Level of achievement: progress made in Granada, June 2008 (M44). Level of achievement. Discussed at AGM (absence of Spanish partners) and in Granada, Spain (M44), but delay in recruiting research assistant for LSHTM 36 has restricted progress.

WP LEI 5 – Data management and cross disciplinary modeling

Person-month inputs are approximate because the PMR of an institute mixes P-Ms from different subprojects.

Work package number	LEI WP5	Start date or starting event:				37
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	1	2	0.5	0.75	2	2	1
Participant id	UB	UM1	UoC				Total
Person-months / participant	1	0.5	1.25				12

Objectives

The general objective is to generate current disease and vector risk maps on the basis of statistical models using pan-European remotely sensed and ground measured eco-climatic predictor data sets.

The specific objective is to collate all geo-referenced databases, and to analyse them to associate the distribution of leishmaniasis in the study regions with climate & environmental descriptors and the distributions of leishmaniasis and sandfly species. In months 25-42, the SP and HITS will collaborate more fully for some preliminary regional and Europe-wide modelling.

Work performed during previous reporting periods

In the first two years of EDEN (2004-6), we started multivariate analyses of the geo-referenced databases (WP1-4) and the modelling of the data from one of the three detailed study regions (in the French Pyrenees), to establish any significant associations (spatial and temporal) between leishmaniasis prevalence, sandfly vector densities and climate & environmental parameters. At the end of these preliminary studies, integrated activities with HITs started to be planned. Most teams attended workshops for the manipulation of datalayers with ArcView software.

In 2006-7, additional GIS training was provided for those teams seeking it (Barcelona, M30). New spatial analyses were restricted to 1) sandfly density or absence/presence in France in relation to climate (NHM 34 with LR Spatial Modelling HIT) and environment (NHM 34 with Environmental Change HIT), and 2) prevalence of canine leishmaniasis in relation to climate and environment (LSHTM 36). Canine leishmaniasis was not associated with vector density or absence/presence. Collaborative research was started with the Environmental Change and Modelling HITs, working with sandfly and environmental data from France, including analysis and planning sessions in Antalya (M27), Leuvan (M30) and London (M36). Progress was shared with other partners at AGM 2007 (M27), Barcelona workshop (M30) and Piacenza workshop (M31).

Description of work

In the next 18 month period (M37-54), the focus will be building on the collaborations started with the HITS to develop models of the spatial & temporal associations between leishmaniasis prevalence, its vectors, and changes in climate & environmental descriptors. The models will be both statistical (in collaboration with LR Spatial Modelling and the Environmental Change HITs) and biological (in collaboration with the Mathematical Modelling HIT).

Achieved results: Sandfly and canine leishmaniasis data for France (partner NHM 34) was given to Mathematical Modelling HIT in January 2008, and further sandfly data provided in September 2008. Preliminary results and conclusions have been received, but a new timetable is required and a geographer may no longer be available to provide all the necessary datalayers.

Deliverables

D LEI12 – The first preliminary model for 3 European regions of the spatial & temporal associations between leishmaniasis prevalence, its vectors, and changes in climate & environmental descriptors (M36). Level of achievement: The long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated.

D LEI19 – The first preliminary model for all regions of the spatial association between leishmaniasis prevalence, its vectors, and climate & environmental descriptors (M42). Level of achievement: The long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated.

Milestones and expected result

M LEI 21 - Workshop to train all partners (M27). Level of achievement: this and other workshops completed, but more help required.

M LEI 26 - Preliminary spatial model outputs are available (M42). Level of achievement: The long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated.

Workpackages WNV1 to WNV5: West Nile virus

Deliverable review

Late payment of funds caused significant difficulties for some teams, but nearly all were able to continue their work without funding from other projects.

Work package	Deliverable number	Deliverable description	Programmed delivery month	Effective delivery month	CIRAD	UNISAP	IZS	IPP	CNM	EID	IVB	ISS	NIRDMI	DDNI
					1	6	7	8	9	10	11	12	43	44
					France	Italy	Italy	France	Spain	France	Czech	Italy	Romania	Romania
WP 1	D WNV-01	Dossiers of available information, preliminary description of study areas, and practical information for decisions on study sites and study routines.	18	18	F	NA	B	NA	NA	F	F	NA	F	F
WP 1	D WNV-16	Comprehensive data set in a common format.	26	26	C	NA	A	NA	A	C	A	A	A	C
WP2	D WNV-04	Seasonal profiles of adult mosquitoes at 1-2 week intervals, coupled with a daily record of climate variables.	30	30	F	NA	F	NA	F	F	J	NA	F	F
WP 2	D WNV-05	Seasonal profiles of ornithophilic and horse-biting activity at 1-2 week intervals, coupled with a daily record of climate variables.	30	30	NA	NA	F	NA	A	F	J	NA	F	NA
WP 2	D WNV-06	Tests for WNV in collections of ectoparasites associated with birds—resident and migratory—captured for serosamples.	30	30	I	I	I	NA	A	NA	NA	NA	I	I
WP 2	D WNV-07	PCR results for WNV in bird ectoparasites for sero-negative and seropositive birds.	30	30	I	I	I	5	NA	NA	NA	NA	I	I
WP 2	D WNV-08	PCR results for WNV in over-wintering *Culex pipiens* populations.	20-30	30	NA	NA	NA	NA	NA	NA	I	NA	I	NA
WP 2	D WNV-09	Survival rates of over-wintering *Cx. pipiens.*	20-30	30	NA	NA	NA	NA	NA	NA	A	NA	A	NA
WP 3	D WNV-17	An estimate of flavivirus seroprevalence in the human population.	12-30	48	NA	NA	NA	NA	NA	NA	A	NA	A	NA
WP 4	D WNV-11	Reports on seroprevalence of WN antibodies by species, sex, age, habitat and season.	30	30	F		F	A	J	NA	J	NA	F	F
WP 4	D WNV-12	Reports on seroprevalence and sero-conversion rates in horses.	30	30	F	NA	F	NA	NA	NA	NA	NA	F	NA
WP 4	D WNV-13	Preliminary data and analysis of seasonal variations in composition and abundance of the avifauna in selected areas.	30	30	F	NA	F	NA	NA	NA	J	NA	F	F
WP 4	D WNV-14	Preliminary data and analysis of the role of birds in introduction and diffusion of the West Nile virus.	30	30	B	NA	B	NA	NA	NA	A	NA	A	C
WP 5	D WNV-15	Preliminary epidemiological analysis of the first two complete transmission seasons (2006 and 2007).	30	30	A	NA	A	NA	NA	A	A	NA	A	A

LEGEND		
ACTION	A	Preparatory phase/some progress
	B	Good progress
	C	Excellent progress
	D	Sources Identified / Requests submitted
	E	Part season of field data
	F	Full season of field data
	G	All problems solved, ready to start in new season
	H	Large body of information and data collected
	I	Samples collected, diagnostic tests in progress
	J	Some data acquired, but agreed protocol not followed
	NA	Not applicable
PROBLEMS	1	Transportation and other resource problems
	2	Licensing and other admin issues
	3	Meeting/workshop postponed
	4	Laboratory closed for renovation
	5	Diagnostic problems to be checked (e.g. false positives)

Table WNV-01. Deliverable status per WNV

Executive summary

Laboratory experiments can begin with strict protocol and a carefully planned time line, but field studies can go on forever! On the whole all teams have lived up to expectations, and there are large amounts of data to collate, to analyse and to publish, yet nature always makes us regret the end of field projects—next year may be so different from the past four. Nevertheless, 2008 has been a bumper year for results, some of them exciting. Dealing in turn with work-packages:

- in WP1, data has been added, but only three teams have made major advances in assembling them in a common data-base; there is an urgent need for the others to follow.
- For WP2, the species succession has been well documented for another year, and the differences and similarities between regions, in the perspective of climatic factors, are most interesting. For example, the Romanian mosquito fauna has been atypical for a third year, ostensibly because of drought conditions, whereas the French fauna has been relatively "normal" in a year with "normal" weather. The observation of a high survival rate of overwintering mosquitoes in the Danube Delta region is most interesting, and will benefit by repetition in the 2008-09 winter. In addition, if confirmed, the RAMP-postive pools of nulliiparous overwintering *Culex pipiens*, will be a major finding. The epidemiological significance of such positives may be more apparent in the context of our findings on species succession under different climatic conditions, and a review of climatic conditions/WNV incidence in the past.
- The plan for a serosurvey of humans (WP3) in regions of transmission was ambitious and proved impractical, though might have been feasible if there had been a significant outbreak of disease in humans. All that can be said is that clinical cases of West Nile virus among humans remain very rare, less than 10 cases over the four years of the project. Indeed, to date, the only major epidemics in the Old

World have been in conditions of inadequate sanitation in substandard buildings in ex-Soviet bloc countries. The latter is a worsening problem in those countries (Marina Soklovola, personal communication), and needs to be addressed (an attempt to obtain funding for a study of mosquito control in such buildings from another source was unsuccessful).

- In WP 4, seroprevalence among birds has continued low except in Spain and Romania, sites of major migratory routes to and from Africa. On the other hand, the considerable outbreak of clinical cases in horses in NE Italy may confirm that transmission can flare up in well-defined localities while seroprevalence remains low in neighbouring areas. Thus negative results (low seroprevalence) are as valuable as positive evidence of transmission, and the concept of EDEN-WN as a comparative study is well-justified.
- Finally, WP5 remains an urgent priority; modelling by CIRAD and DDNI will only be truly valuable when the same techniques are applied to data from the other teams.

Publications

The following PhD students have completed their thesis at least partially using EDEN data:

None.

The following PhD students are currently conducting research using EDEN data:

Liviu Florian, NIRDMI. Entomological studies in Danube Delta and Bucharest. Romania. Seasonality of bird-biting mosquitoes; identification of putative vectors; tests for virus in mosquitoes; overwintering of mosquitoes; possible vertical tranmission in overwintering mosquitoes; collaboration with DDNI ornithologists and Gregory Lambert, EID.

Gregory Lambert, EID. Entomological studies in Rhône Delta (Camargue). Seasonality of bird-biting mosquitoes; identification of putative vectors; overwintering of mosquitoes; collaboration with CIRAD ornithologists and Liviu Florian (NIRDMI.

The following scientific documents have been published by EDEN-WNV team members. Those marked by an EDEN publication number were officially validated by the EDEN Steering Committee. those marked by EDENXXX were supported at least partially by EDEN funds but were submitted for validation to the EDEN SC after publication (no EDEN number included in final paper).

Publications of EDEN-WNV (with an EDEN number)

- FIGUEROLA, J., SORIGUER, R., ROJO, G., TEJEDOR, C. & JIMENEZ-CLAVERO, M. 2007. Seroconversion in wild birds and local circulation of West Nile virus, Spain. Emerging Infectious Diseases, 13: 1915-1917. EDEN0045
- LÓPEZ G, JIMÉNEZ-CLAVERO MA, TEJEDOR CG, SORIGUER R, FIGUEROLA J. (2008). Prevalence of West Nile virus neutralizing antibodies in Spain is related to bird migratory behaviour. **Vector-Borne and Zoonotic Diseases** 8(5):615-621. EDEN0079
- HUBÁLEK Z., WEGNER E., HALOUZKA J., TRYJANOWSKI P., JERZAK L., ŠIKUTOVÁ S., RUDOLF I., KRUSZEWICZ A.G., JAWORSKI Z., WLODARCZYK R. (2008): Serologic survey of potential vertebrate hosts for West Nile virus in Poland. *Viral Immunol.* 21: 247-254. EDEN 0080
- HUBÁLEK Z., HALOUZKA J., JUŘICOVÁ Z., ŠIKUTOVÁ S., RUDOLF I., HONZA M., JANKOVÁ J., CHYTIL J., MAREC F., SITKO J. (2008): Serologic survey of birds for West Nile *Flavivirus* in southern Moravia (Czech Republic). *Vector-Borne Zoon. Dis*. 8: 859-866. EDEN 0085
- FIGUEROLA, J., JÍMENEZ-CLAVERO, M.A., LÓPEZ, G., RUBIO, C., SORIGUER, R., GÓMEZ-TEJEDOR, C. & TENORIO, A. (2008). Size matters: West Nile Virus neutralizing antibodies in resident and migratory birds in Spain. **Vet Microbiol.** 132(1-2):39-46. EDEN0095
- HALOUZKA J., JURICOVA Z., JANKOVA J., HUBALEK Z. (2008): Serologic survey of wild boars for mosquito-borne viruses in South Moravia (Czech Republic). *Vet. Med.* (Czech) 53: 266-271. EDEN 0104
- BALANÇA, G., GAIDET, N., SAVINI, G., VOLLOT, B., FOUCART, A., REITER, P., LELLI, R., MONICAT, F. Low West Nile virus circulation in wild birds in an area of recurring outbreaks in Southern France. (Submitted to Vector-borne and Zoonotic Diseases, EDEN0113).

- ŠEBESTA O., RETTICH F., MINÁŘ J., HALOUZKA J., HUBÁLEK Z., JUŘICOVÁ Z., RUDOLF I., ŠIKUTOVÁ S., GELBIČ I., REITER P.: First record of *Anopheles hyrcanus* (Diptera: Culicidae) in the Czech Republic. *Med. Vet. Entomol.*, submitted. EDEN 0119
- RUDOLF I., MENDEL J., HUBÁLEK Z., HALOUZKA J., ŠIKUTOVÁ S., JUŘICOVÁ Z.: Detection of West Nile virus RNA in overwintering Culex pipiens mosquitoes, 2007, Central Europe. Prepared for *Emerg. Inf. Dis.*
- ROCHE, B., ELGUERO, E., BALANÇA, G., MORAND, S., GUÉGAN, J-F., GAIDET, N. Disentangling effects of intrinsic competence and ecological factors for epidemics patterns: The case of West Nile fever. (In preparation).
- L'AMBERT, G, FERRÉ, JB., SCHAFFNER, F., PASTEUR, N., FONTENILLE, D. Comparison of different trapping methods for mosquito monitoring in the Rhône Delta. (In final draft).
- Seasonal dynamic of mosquitoes in the Rhone delta: in preparation, will be submitted at the end of January/beginning February
- Host preferences of potential vector of West Nile virus in the Rhone Delta (summer 2009)

Publications related to EDEN-WNV (without an EDEN number)

- FIGUEROLA, J., JÍMENEZ-CLAVERO, M.A., ROJO, G., GÓMEZ-TEJEDOR, C. & SORIGUER, R. (2007). Prevalence of antibodies specific against West Nile virus in colonial aquatic birds in southern Spain. **Avian Pathology** 36(3) **:** 209- 212
- JIMENEZ-CLAVERO, M.A., GOMEZ-TEJEDOR, C., ROJO, G., SORIGUER, R. & FIGUEROLA, J. (2007). Serosurvey of West Nile virus in equids and bovids in Spain. **Veterinary Record** 161**:** 212.
- MARINOV, M. JR, KISS, J., RAILEANU, S., CONDAC, M., DOROFTEI, M., PRIOTEASA, F., ALEXE, V. (2007) Preliminary results regarding research of West Nile Virus on Danube Delta Biosphere Reserve. Scientific Annals of the Danube Delta National Institute. Editura Tehnica. 13: 59 – 66.
- PRIOTEASA, F., FALCUTA, E., MARINOV, M. JR., NICOLESCU, G. (2007) Mosquitoes (Diptera: Culicidae) in Mila 26 – Maliuc area (Danube Delta, Romania) – preliminary data. Scientific Annals of the Danube Delta Institute. Editura Tehnica. 13: 89 – 96.
- MARINOV, M. jr. (2006). Emerging Diseases in a Changing European Environment (EDEN) – general presentation and DDNI team's contribution. Scientific Annals of the Danube Delta National Institute. Editura Tehnica. 12: 75 – 82.
- COIPAN, E., MARINOV, M. JR., ARSENE, M., VLADIMIRESCU, A. (2006). Study of ticks (Acari: Ixodidae) in Danube Delta and Tulcea district in 2005. Scientific Annals of the Danube Delta National Institute. Editura Tehnica. 12: 13 – 18.

Meetings where papers involving EDEN-WNV ideas have been presented

ROMANIA, BUCHAREST: NIRDMI

- Oral presentation "Investigation of the West Nile virus circulation in Romania"(Nicolescu et al.) at The 2nd International Conference of Economic Entomology, Cairo, Egypt, December 2007
- Oral presentation "Ornithophilic mosquitoes (Diptera: Culicidae) in the Danube Delta Biosphere Reserve and their possible role in the transmission of West Nile virus (Flaviviridae)" (Prioteasa et al.) at EDEN Annual Meeting, Brno, Czech Republic, January 2008
- Poster "The transmission cycles of West Nile virus in Romania and the detection of virus presence" (Purcarea–Ciulacu et al.) at The 13th International Congress on Infectious Diseases (ICID), Kuala Lumpur, Malaysia, June 2008
- Poster "Evaluation of the re-emergence risk of mosquito-borne diseases in Romania" (Nicolescu et al.) at The 23rd International Congress of Entomology, Durban, South Africa, July 200
- Poster "Investigations on the mosquito fauna (Diptera: Culicidae) in the Danube Delta Biosphere Reserve for the identification of the vector species of West Nile virus (Flaviviridae)" (Prioteasa et al.) at The 16th National Conference of Parasitology with international participation, Mamaia, November 2007
- Poster "A preliminary pattern about West Nile virus transmission in the Danube Delta" (Prioteasa et al.) at The 18th Symposium "Deltas and Wetlands", Tulcea, Romania, September 2008

Presentations and posters DDNI

- MARINOV, M. JR., KISS, J. Emerging Diseases in a Changing European Environment (EDEN) – DDNI team's contribution. Poster presented at the EDEN Annual Meeting, Montpellier, France, 14 – 17 of February, 2005.
- MARINOV, M. JR., KISS, J. (2005) Emerging Diseases in a Changing European Environment (EDEN) – DDNI team's contribution. Poster presented at the Danube Delta National Institute International Symposium (Deltas and Wetland), Tulcea, Romania, 28 September – 2 October, 2005.
- PRIOTEASA, L., PURCAREA-CIULACU, V., VLADIMIRESCU, A., COIPAN, C., DUMITRESCU, G., FALCUTA, E., MARINOV, M. JR., NICOLESCUL, G. (2005). The mosquito fauna in the Danube Delta and lagoons and in Bucharest area in 2005. Poster presented at the EDEN Annual Meeting, Rovaniemi, Finland, 12 - 14 of January, 2006.
- MARINOV, M. jr. (2006). The ornithofauna of Danube Delta Biosphere Reserve and its implication on West Nile Virus transmission. Poster presented at the EDEN Annual Meeting, Rovaniemi, Finland, 12 - 14 of January, 2006.
- MARINOV, M. jr., Kiss, J. (2006). Preliminary results regarding research of West Nile Virus on Danube Delta Biosphere Reserve. Poster presented at the Danube Delta National Institute International Symposium (Deltas and Wetland), Tulcea, Romania, 21 - 23 septembrie 2006.
- PRIOTEASA, F., FALCUTA, E., MARINOV, M. jr. (2006). Ecofaunistical Survey on Mosquitoes in Maliuc – Mila 26 (Danube Delta, Romania) – preliminary data. Poster presented at the Danube Delta National Institute International Symposium (Deltas and Wetland), Tulcea, Romania, 21 - 23 septembrie 2006.
- MARINOV, M. JR., KISS, J. (2007). Preliminary results regarding research of West Nile Virus on Danube Delta Biosphere Reserve. Poster presented at the EDEN Annual Meeting, Antalya, Turkey, 8 - 13 of January, 2007.
- MARINOV, M. jr. (2007). The Ornithofauna of Danube Delta Biosphere Reserve and its implication on West Nile Virus transmission. Power Point Presentation at the EDEN PhD Meeting during the EDEN Annual Meeting, Antalya, Turkey, 8 - 13 of January, 2007.
- FALCUTA, E., PRIOTEASA, F., NICOLESCU, G., MARINOV, M. jr. (2007). A comparative survey of the anopheline (Diptera: Culicidae) fauna from the maculipennis group between three areas belonging to the Danube Delta Biosphere Reservation – preliminary data. Poster presented at the Danube Delta National Institute International Symposium (Deltas and Wetland), Tulcea, Romania, 20-22 septembrie 2007.
- MARINOV, M. JR., KISS, J. (2008). Preliminary results regarding research of West Nile Virus on Danube Delta Biosphere Reserve and Dobrudja Tableland - Romania. Poster presented at the EDEN Annual Meeting, Brno, Czech Republic, 14 - 18 of January, 2008.
- MARINOV, M. jr. (2008) The ornithofauna of Danube Delta Biosphere Reserve and its implication on West Nile Virus transmission (II). Power Point Presentation at the EDEN PhD Meeting during the EDEN Annual Meeting, 14 - 18 of January, 2008.
- MARINOV, M. jr., Kiss, J. (2008). Preliminary results regarding research of West Nile Virus on Danube Delta Biosphere Reserve and Dobrudjean Tableland – Romania. Poster presented at the IX International Symposium on Vector and Vector Borne Disease, 15-17 February 2008, Puri, Orissa (India). Nat. Inst. of Malaria Research, Delhi – India. Nat. Vector Borne Disease Control Programme, Delhi, India. Nat. Acad. Of Vector Borne Diseases, Bhubaneswar, India.
- PRIOTEASA, F., FALCUTA, E., REITER, P., NICOLESCU, G. (2008). A preliminary Pattern of the West Nile Virus Circulation in Danube Delta. Poster presented at the Danube Delta National Institute International Symposium (Deltas and Wetland), Tulcea, Romania,18 - 20 septembrie 2008.

FRANCE: EID

- Oral communication at the "Journée interrégionale d'information sur les arboviroses", Aix-en-provence, 10/06/08.
- Oral communication on the EDEN and EDEN-WNV projects during a EID meeting, Montpellier, (04/12/08).
- Communication on EDEN activities in EID's newspaper, communicated to 216 local authorities in 21 departments, in metropolitan France and French overseas department.

FRANCE: IP
Oral presentations:
- "Lutte Antivectorielle [Vector control]". Meeting organized by Ministères de l'Ecologie, du Développement et de l'Aménagement Durable, de l'Enseignement supérieur et de la Recherche, de l'Agriculture et de la Pêche, de la Santé, de la Jeunesse et des Sports, et la secrétaire d'Etat chargée de l'Ecologie, Montpelier, June.
- Epidemiology and control of West Nile virus in Europe and the Americas. Lecture given [in French] to IRD Master's course, Ouidah, Republic of Benin.
- Meeting on West Nile Virus organized by the Fondazione Iniziative Zooprofilattiche e Zootecniche in Brescia, Italy
- Lecture on West Nile virus to veterinarians in oubreak zone, Brescia, Italy.

SPAIN : IVB
- S. Ruiz Contreras, F. Cáceres Benavides, R.C. Soriguer Escofet, J. Figuerola Borras, S. López Sánchez, 2008. El virus West Nile en vectores y reservorios en Andalucía. Comunicación oral invitada XI Congreso Nacional de Seguridad Ambiental. Sevilla, España Año: 2008
- Figuerola, J., Jiménez-Clavero, M.A., López, G., Rubio, C., Soriguer, R., Gómez-Tejedor, C., Tenorio, A. 2007 Relationships between evolutionary and ecological factors and the prevalence of West Nile virus antibodies in birds. Com Oral. Bird migration and global change. Libro de resúmenes del Congreso, Algeciras, España. AÑO: 2007.
- Agüero, M., San Miguel, E., Gómez-Tejedor, C., Jiménez Clavero, M.A., 2007. Detección molecular de virus animales a gran escala: impacto en el control de enfermedades emergentes. Póster CONGRESO: IX Congreso Nacional de Virología. Lbro de resúmenes del Congreso Zaragoza. AÑO: 2007
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- Jiménez Clavero, M.A., Soriguer, R., Gomez-Tejedor, C., López, G., Rojo, G., Figuerola, J., 2007. Survey of West Nile virus-specific antibodies in wild birds in Southern Spain: Póster, 5th Internacional Conference on Emerging Zoonoses. Libro de resúmenes del Congreso. Limassol, Chipre AÑO: 2007
- Figuerola, J. y R.C. Soriguer. 2007: Prevalence of West Nile virus neutralizing antibodies in birds: Separating evolutionary from ecological factors. Comunicación oral. Annual Meeting of the Waterbird Society. Barcelona.
- Figuerola, J., M. A Jiménez y R.C. Soriguer. 2007 Relationship between evolutionary and ecological factors and the prevalence of West Nile virus antibodies in birds. Comunicación oral invitada. Bird Migration and Global Change. Algeciras, España. Master in Cambio Global y Enfermedades Emergentes. Palma de Mallorca . Nov 2008.

WP WNV 1 – Landscapes, biotopes and habitats

Work package number	WNV1		Start date or starting event:			37		
Participant id		IZS	DDNI	NIRDMI	EBD	EID	IVB	CIRAD	Total
Person-months / participant		4	4	4	4	4	4	5	29

Objectives

The fundamental approach of EDEN-WNV is gain insight into the variables favourable for epizootic/epidemic transmission of the virus through a systematic comparison of environmental parameters in all the study areas (the « comparative approach » presented in the original proposal).

Work performed during previous reporting periods

All participants have now gathered an impressive compendium of historical and environmental information pertinent to their study areas. Much of this is available in great detail and covers an extended time span (nearly 600 years for one site in Czech Republic!). The number of records of WNV in Romania over the past half century is particularly impressive, and, combined with serological data, detection of virus in birds and mosquitoes, and clinical records points to the Romanian study sites as having the highest level of epizootic transmission. It appears that this is only matched by enzootic/epizootic transmission in Spain, though human infections are less common. It is therefore highly desirable that NIRDNI and EBD make a special effort to co-ordinate their efforts in the context of WP1 and WP5. That is not to say that IZSAM/ISS and IVB data has less importance; on the contrary, their large databases, both in the field and the laboratory, will be a key component in the final, comparative analysis.

Ecological mapping surveys were pursued in the different countries and study sites, both for birds and mosquitoes. See section 2 'Work package progress of the period" in this report for more details.

Description of work

Target dates are hard to set because progress has not been uniform for all participants, but the collaborative efforts between CIRAD/EID/DDNI, and CIRAD/IZSAM, have reached an encouraging stage and will serve as a model for efforts by the remaining participants. Discussion on how to energize the process will be a priority at the Annual Meeting in Brno in January 2008. At a later stage, all groups will meet with the CIRAD HIT squad in Montpellier, after which HIT team members should spend significant time with each participant in situ; this is the only way that the work can be replicated across the geographic span of the project. Co-ordination with the Africa platform must also be a priority; hypotheses on the natural history of enzootic/epizootic transmission can only be meaningful by consideration of both ends of the migratory pathways. The co-ordinator will foster additional communication with institutions in countries outside the EDEN network (e.g. Algeria. Tunisia. Russia. Israel).

Achieved results:

ITALY: IZSAM and ISS

In collaboration with the HIT RS (Horizontal Integration Team on Remote Sensing tools) (CIRAD, partner n°1), in 2006, the study area was investigated and classified in ecological areas through a satellite image processing. To provide a land cover map of the study area, a satellite ASTER image (15 m resolution) was classified into different ecological units relevant for entomological and ornithological surveys. The typology of the map was defined during a field mission, to identify the land cover types present in the study area and to sample training sites required for the image processing. Following field activities performed at the beginning of 2007, an update of the land cover map based on the ecological units classification already mentioned appeared necessary, due to the vast differences in water level in the marsh area in different seasons. To perform this new classification, the HIT RS was contacted again and work is underway. Based on the available land-cover maps of the study area, in 2007 a bird census (still underway) and specific mosquito trappings were performed to deepen the knowledge of the bird and mosquito species present in the same ecological units. This activity will enable to acquire useful data for modeling activities to perform in the forthcoming reporting periods. The ecological map is therefore the same of the previous reporting period, and so are cartographic projections and formats.

ROMANIA. BUCHAREST: NIRDMIC
Climate - 2008

After the extremely droughty year 2007 (record for the last 100 years), the precipitations were low during the winter 2007-2008 in the eastern and southern part of Romania outside the Carpathian Arch. The spring was wet, but the resumption of the life cycles by mosquitoes was later than usually because of the low temperature and the very small number of the over-wintering mosquitoes. At the end of the spring, there were high rainfalls within very short periods of time (only 15-30 min) which have washed the larval habitats and destroyed the larvae. Very high temperatures and the lack of rains in summer and in September maintained the drought and the very low populations of mosquitoes at least in the south of the country. These climatic conditions influenced the mosquito fauna and the WNV circulation in some areas and moments. Daily climatic data (min, mean and max temperatures, quantity of precipitations, relative humidity, cloudiness and wind speed) in Bucharest metropolitan area and Tulcea district (Tulcea and Sulina) from the National Meteorological Administration for 2008 have been obtained and analysed.

Study sites
Because the last human cases of WNV infections in Bucharest metropolitan area have been registered in 2004 and 2005, and in Tulcea district in 2001, and the mosquito fauna remained extremely low in 2008 in the especially in these areas, we have extended the area of investigation of WNV circulation in birds, horses, and mosquitoes. We have made investigations in an area south of Bucharest in the Danube Valley (included in the Bucharest metropolitan area) and in other districts of the Romanian Plain. Description of the new sites is in progress.

FRANCE: CIRAD
In 2006, common ecological units have been defined for ornithological and entomological surveys in the Camargue area. A primary bird data base was built according to expert opinion and literature, describing variation in species occurrence in time and space for 180 bird species over the entire study area. In 2007, this database has been completed and improved and field validation was conducted using a standard bird census technique (point counts) to evaluate the relative occurrence of various bird species in various habitats, and to compare the diversity and the abundance of birds between different ecological units. In 2008, the bird database has been assessed in relation to the results of the bird censuses and statistical tests were conducted. These tests revealed that the data-base was consistent. It has been integrated in a GIS including habitat and mosquito data, as well as ecological and behavioural data on birds. Different scenarios of introduction and spread of WNV in the Camargue area will be tested next year with this GIS and a scientific paper will be written. Another data-base containing ecological and behavioural data on American birds has been built to test for the importance of ecological factors and reservoir competence of birds in WNV spread in North America. A manuscript has been written (see list).

CZECH REPUBLIC: IVB
Hydrological data (Thaya River). New meteorological data (up to the end of 2007) from the study areas were sent to the coordinators of TBD and WNV.

SPAIN: EBD
Data on climatology, flooding, water table level, and vegetation in the study area were obtained and incorporated in the data-base.

ROMANIA. DANUBE DELTA: DDNI

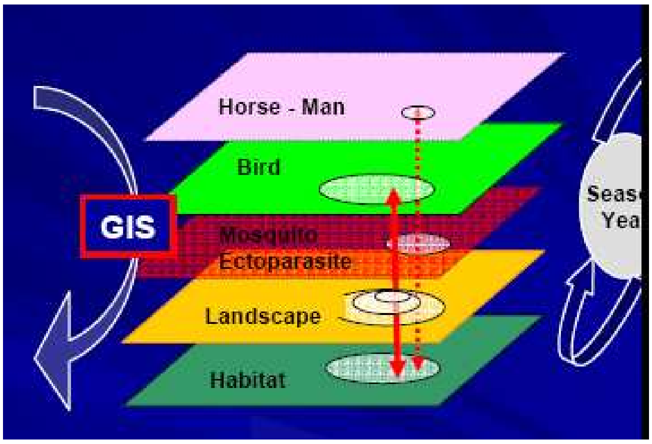


Fig. WNV01 - Characterization of the diversity of reservoir – vector associations in space and time

We have maintained close contact with CIRAD and EID to standardize database for entomology, ornithology and teledetection, and applied this to the habitats and conditions of Danube Delta Biosphere Reserve and Dobrudja Tableland. Our colleagues from Cantacuzino Institute will use for database integration as a background, the DDBR Vegetation Map and Bird Habitat Map, both on Arc View format. As soon as we obtain the pattern of characterization of diversity of horse reservoir associations in space (CIRAD), we start to work on it. For ecological and landscape approach, it has been established that all EDEN-WNV teams must do the characterization of the diversity of reservoir – vector associations in space and time. Three layers (habitat, landscape and bird) were completed in 2007 (Fig. 1). The mosquito layer is in progress and we (DDNI and NIRDMIC) will soon start working at the last layer (Horse – Man) in collaboration with CIRAD.

Deliverables

D WNV 16 – Comprehensive data set in proper format (M26). IZS, DDNI and CIRAD are done. Other teams are still in early stages although they have assembled large amounts of data in other formats

Milestones and expected result

M WNV18 - Completed dossiers of data and other information. and a comprehensive data set in a format that is common to all contractors (M26). IZS, DDNI and CIRAD are ahead on this, others need to follow.

WP WNV 2 – Vector bionomics and competence

Work package number	WNV2	Start date or starting event:		37			
Participant id	IZS	DDNI	UNISAP	NIRDMI	CNM	EID	IVB
Total per participant	24	12	6	42	42	24	30
Participant id	EBD	IPP	ISS				Total
Total per participant	18	4	9				211

Objectives

The principal objective of WP WNV2 is to identify potential WNV vectors (mosquitoes) in Europe, obtain maximal information on their ecology and seasonal activity, assess whether ectoparasites of resident and migrant birds may serve as reservoir of virus between mosquito seasons, and determine whether WNV is able to over-winter in Europe.

Field science requires much more time than laboratory science! Arbovirus transmission is seasonal and a useful data set can only be gained with several season's work. This is particularly true for EDEN because we seek associations between environmental change, such as climate; signatures of such associations can only be obtained by comparing incidence between years. For this reason, in addition to the historical data that has been accumulated, a third year is highly desirable, particularly for those teams that did not get a good start in 2005. Moreover, despite the promising accord achieved at the workshop, several teams did not follow the agreed protocol either in part or in full. The fundamental principle of the whole WNV project is that it is a comparative study. Therefore we must insist that all teams comply with the protocol. Of course, additional studies are highly desirable, but these must be secondary to the overall project.

For these reasons, during the third 18 months, the specific objectives will continue as before*, i.e.* to:

- Monitor seasonal abundance of ornithophilic mosquitoes, using bird-baited traps
- Monitor short- and long-term weather fluctuations in parallel with numbers and species of mosquitoes collected in bird-baited traps
- Identify mosquito species likely to serve as "bridge vectors" between birds/horses and birds/humans and test for vector competence and vertical transmission.
- Determine whether WNV is present in overwintering *Culex pipiens*
- Test for WNV in ectoparasites of birds

Work performed during previous reporting periods

Seasonal abundance was monitored by regular trapping of mosquitoes in Camargue (France), urban and peri-urban Bucharest and Danube delta in Romania, La Doñana National Park (Spain), Czech Republic, Italy. Traditional entomological parameters have been recorded (longevity of females evaluated by ovarian dissection, identification of blood meals…). Some of theses samples were already tested for flavivirus/WNV by RT-nested-PCR but no isolation yet. Ectoparasites were collected on from birds trapped for ringing (WP WNV 3).

Participants had to tackle a plethora of preparatory tasks and problems: permission to work in conservation areas and to bleed wild birds, administrative requirements of parent establishments and national governments, formal agreements with non-EDEN groups working on WNV in the same regions, several others including new emerging diseases (avian influenza…).

Description of Work

(a) Potential vectors. All but one participant has completed one or more full seasons of bi-weekly mosquito captures with bird-baited traps and CO2 traps. The seasonal profiles that have emerged, and their species composition, give a fair idea of what may be expected in a "normal" year, and which ornithophilic species are likely to be the principal vectors of WNV etc. One exception may be Romania, where two years of exceptional spring/summer rainfall were followed (2007) by a year of severe drought.

The protocol adopted by the EID team - three series of intensive trapping episodes, in spring, summer and autumn, centered on selected biotopes - will replace bi-weekly collections. In addition to a reduction in transport costs (for most participants), it will allow more emphasis on laboratory work during the intervening periods (e.g. determination of parous rates, and, if possible, number of gonotrophic cycles completed).

The RAMP kit by the DDNI/NIRDMI teams has proved highly satisfactory. All participants will use this technology, with modification of the recommended protocol to enable confirmation of positive tests by virus isolation.

Lastly, it is hoped that contacts with Algerian and Tunisian teams will enable them to use EDEN protocols at sites where large numbers of birds congregate after their trans-Saharan flight.

Achieved results:

ITALY: IZSAM and ISS

a) Potential vectors and **d) Horse-biting species**

Catch sites selected for the entomological activity in the study area in 2008 are the same 5 of 2007 and 2006: 4 of these are located inside horse farms and only one inside the protected area of the Padule di Fucecchio (Righetti-La Monaca). As agreed following the discussions in the annual meeting 2008, catches have also been performed near ornithological catching sites in Padule di Fucecchio (Le Morette). Catch sites were selected considering the following criteria:

- different ecological characteristics;
- outbreaks of disease/viral circulation in 1998;
- seroconversion in horses in 1998 and the following years;
- presence of horses and/or birds;
- entomological catches in 2005;
- owner’s availability and collaboration.

As no statistically meaningful differences were recorded in 2006 between the numbers of mosquitoes collected with the same type of trap at different height, only ground level traps have been run in 2007 and 2008. In 4 sites, 2 traps were placed to capture mosquitoes: one bird-baited trap at ground level (about 1.5 m of height), and one CO2 baited trap at ground level (about 1.5m of height). Aspiration of mosquitoes from resting sites (shelters or boxes for horses, etc.) has also been performed to catch adults. A hygrothermometer was placed in each site and max and min temperature and humidity were recorded during catching. Catches were made on a night fortnightly trapping bases from 18 March to 16 September 2008. An entomological database was created and sent to GIS Database Manager Environment Research Group Oxford.

Table 1. Geographic coordinates of entomological traps in 2007.

Catch sites	Traps	Longitude	Latitude
Borgo a Baggiano (PT)	CO_2-baited trap	43.87743	10.748
	Bird-baited trap	43.87702	10.748
Castelvecchio (LU)	CO_2-baited trap	43.7748	10.612
	Bird-baited trap	43.77261	10.6122
Galleno (FI)	CO_2-baited trap	43.76004	10.7345
	Bird-baited trap	43.76002	10.7347
Monsummano (PT)	CO_2-baited trap	43.84855	10.802
	Bird-baited trap	43.84874	10.8017
Righetti - La Monaca (PT)	CO_2-baited trap	43.84855	10.802
	Bird-baited trap	43.84874	10.8017
Le Morette (PT)	CO_2-baited trap	43.80753	10.81476
	Bird-baited trap	43.80753	10.81476

Table 2a. Entomological catching from 18 March 08 to 21 August 2008. Borgo a Buggiano (PT)

Catch sites	Traps	Species	♀	♂	Total Adults
Borgo a Buggiano (PT)	Bird-baited trap	*Cx pipiens*	106		106
	CO_2-baited trap	*Cs. annulata*	1		1
		Cx pipiens	39		39

Table 2b. Entomological catching from 18 March to 21 August 2008. Castelvecchio (LU)

Catch sites	Traps	Species	♀	♂	Total Adults
Castelvecchio (LU)	Aspiration horses box	*An. maculipennis*	15		15
		Cs. annulata	1		1
	Bird-baited trap	*Cx pipiens*	57		57
	CO_2-baited trap	*An. maculipennis*	24	1	25
		Cs. annulata	9		9
		Cx pipiens	81		81
		Oc. caspius	13		13

Table 2c. Entomological catching from 18 March to 21 August 2008. Galleno (FI)

Catch sites	Traps	Species	♀	♂	Total Adults
Galleno (FI)	Bird-baited trap	*Cq. richiardii*	2		2
		Cx pipiens	767		767
	CO_2-baited trap	*Ae. albopictus*	1		1
		Cq. richiardii	2		2 1

Catch sites	Traps	Species	♀	♂	Total Adults
		Cx pipiens	369		
		Cs. annulata	2		
		Oc. echinus	2		
		Oc. caspius	1		

Table 2d. Entomological catching from 18 March to 21 August 2008, Monsummano (PT)

Catch sites	Traps	Species	♀	♂	Total Adults
Monsummano (PT)	Aspiration horses box	*An. maculipennis*	3	6	9
		Cs. annulata	2		2
		Cx pipiens	16	24	40
	Bird-baited trap	*Cx pipiens*	621		621
	CO_2-baited trap	*Ae. albopictus*	5		5
		Ae. vexans	1		1
		An. maculipennis	4		4
		An. plumbeus	1		1
		Cx pipiens	359	6	365
		Oc. caspius	3		3

Table 2e. Entomological catching from 18 March to 21 Aug. 2008, Righetti-La Monaca Monsummano (PT)

Catch sites	Traps	Species	♀	♂	Total Adults
Righetti-La Monaca Monsummano (PT)	Bird-baited trap	*Cx pipiens*	277		277
	CO_2-baited trap	*An. plumbeus*	2		2
		Cx pipiens	193	2	195
		Oc.caspius	1		1
		Oc.rusticus	1		1

Table 2f. Entomological catching from 18 March to 21 August 2008. Le Morette (PT)

Catch sites	Traps	Species	♀	♂	Total Adults
Le Morette (PT)	Bird-baited trap	*Cx pipiens*	65		65
	CO_2-baited trap	*An. maculipennis*	1		1
		Cx pipiens	234		234

Parous rates (ISS)

In order to collect mosquito females for parity rate and blood meal analysis, we carried out 3 surveys in June, July, and September. This activity was in collaboration between the CeSME of IZS A&M, and the Medical Entomology Unit of ISS, in the same sites than the last year, including an additional catching site (Le Morette), where blood sampling from birds was performed. Parity rate analysis is an empiric method to establish the parous proportion of female mosquitoes as a parameter necessary for the estimation of mosquito longevity, by studying the absence or presence of the coiled tracheolar skeins in fresh ovaries.
The analysis was carried out by Medical Entomology Unit at ISS on *Culex pipiens* only, as the main WN vector species; therefore, we focused on this species because is the most frequent one in the catches by bird-baited trap in order to collect not fed females mosquitoes.
The samples of *Culex pipiens* mosquitoes were removed from the rest of the collected mosquitoes and identified using morphological identification key by microscope dissecting. Ovaries from a portion of female mosquitoes captured by bird-baited traps were dissected to determine parity; when the number of females

caught by such traps was not large enough, females collected in resting sites were also dissected. The dissection of the mosquito abdomens was carried out at Medical Entomology Unit of ISS, according to WHO protocols (WHO, 1975), using at least 20 females mosquitoes from the 6 sites of the study area, to establish the parous/nulliparous proportion. During the study period the parity rates varied showing the physiological increment in middle summer, followed by a decrement in the late summer and in the autumn as are shown in the following table 3.

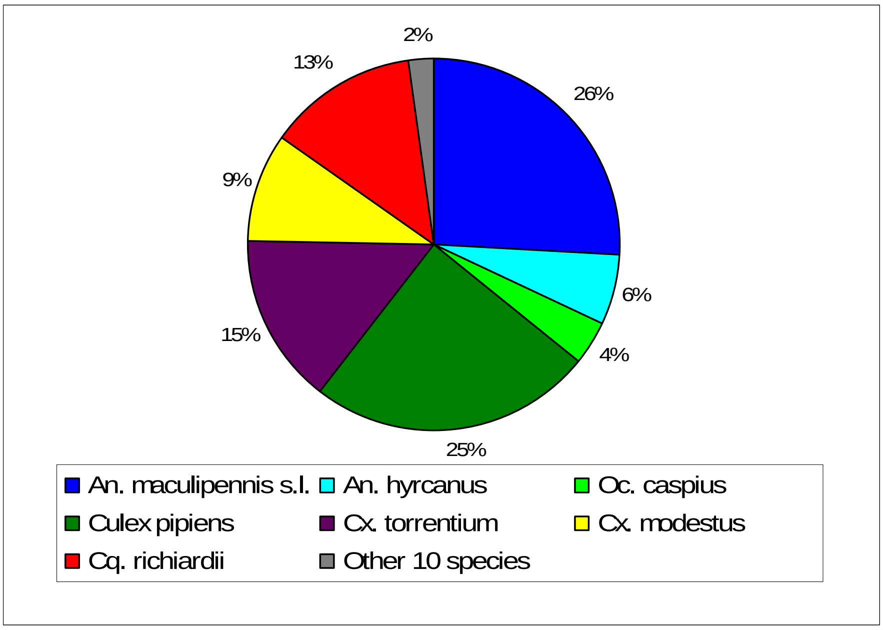
Tab.3 Percentage of parous females in the study area sites

Site	**Date**		
	5/6/08	31/7/08	5/9/08
Giusti	0.30	0.70	0.50
Righetti	0.34	0.83	0.36
Cerbaie	0.26	0.62	0.38
Castelvecchio	0.20	0.66	---
Borgo a Buggiano	0.40	0.64	0.47
Le Morette	---	0.88	0.44

Blood meals analysis
The exam of the blood meals of fed mosquitoes is still ongoing according to the ELISA method direct on nitrocellulose. Fed mosquitoes were caught in animal shelters selected among the ones frequented by different livestock and humans in order to asses the host preference of the species. Blood meals are being tested against horse, dog, bovine, goat, chicken, and human antigens.

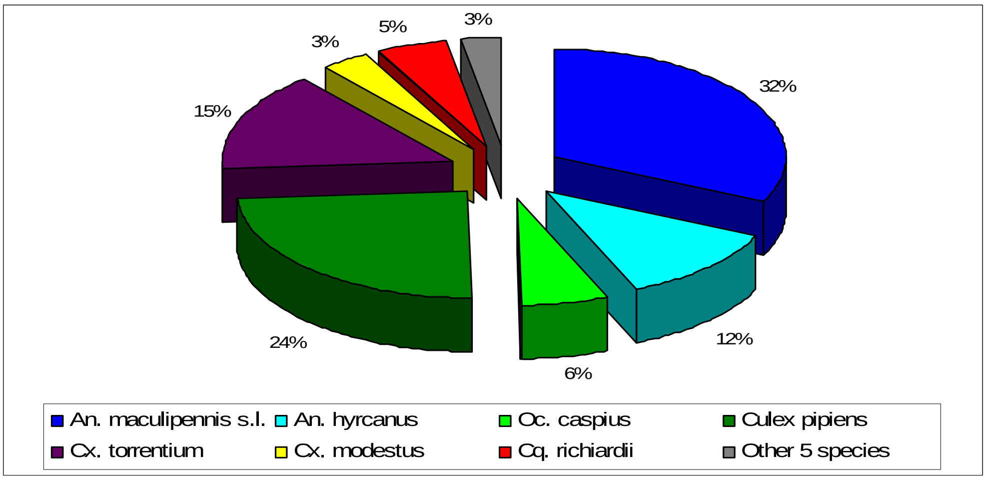
ROMANIA. BUCHAREST: NIRDMIC
The evolution of the climate factors in 2008 had a drastic effect on the mosquito fauna leading to the very low abundance of the mosquito population especially in the south of the country and the total lack of mosquitoes in some areas and moments.

Investigations on mosquito fauna in the Danube Delta and lagoons
Investigations on mosquito fauna in the Danube Delta and lagoons have been made from March to October in Tulcea district at Mila 26 (Maliuc area), Sălcioara and Grindul Lupilor, and in several other areas of the delta (Channel and Grind Stipoc, Raducu and Rotundu Lakes, Crisan and Ghermandi Channels). The mosquitoes (9,816 specimens) were collected on human baits and bird-baited traps. CDC light traps, entomological net (from resting places in vegetation) and with hand aspirators inside the animal shelters, chicken coops and houses. There were also investigations on over-wintering mosquitoes in Tulcea in November 2007 – February 2008 and November 2008.



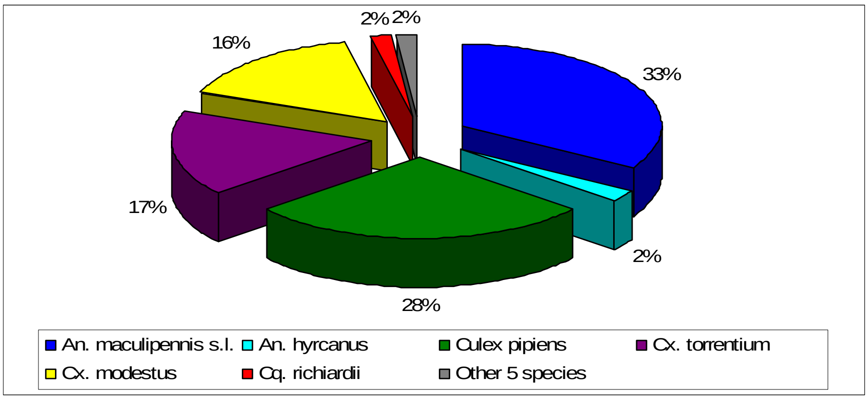
Mosquito species collected in the Danube Delta and lagoon area in 2008

There were identified 17 mosquito species this year in the Danube Delta and lagoons. This number is lower than the total number of 24 species recorded by now in the area. The fauna has been dominated equally by *An. maculipennis s.l.* and *Cx pipiens*. Mosquitoes of the *maculipennis* group dominated as usually in the Delta, and the identified species were *An. atroparvus* and *An. messeae*. The dominance of *Cx pipiens* is unusual for the Danube Delta and lagoon area, but it has been registered in 2007 and this year in Mila 26 and Grindul Lupilor as the effect of the drought even in this areas. This species has usually a quite low presence in the Delta especially in natural habitats which are specific wet areas not so favorable for this species. Because of the drought many former wet areas became dry and *Culex pipiens* larvae developed in small pools remained on the dry soil. *Culex torrentium* was identified in significant proportion in mixed populations with *Cx pipiens*. Although six *Ochlerotatus* species have been found out (9 species are recorded in the area) they were in very low densities. *Oc. caspius* was among the dominant species on extended areas in the Delta but it reached the proportion of only 3.8% among the collected specimens. Only several specimens of *Aedes vexans* were found. The low densities until the missing of spring species of *Aedes* and *Ochlerotatus* genera was a general characteristic of the mosquito fauna in Romania in the last 3 years because of the specific climatic conditions.



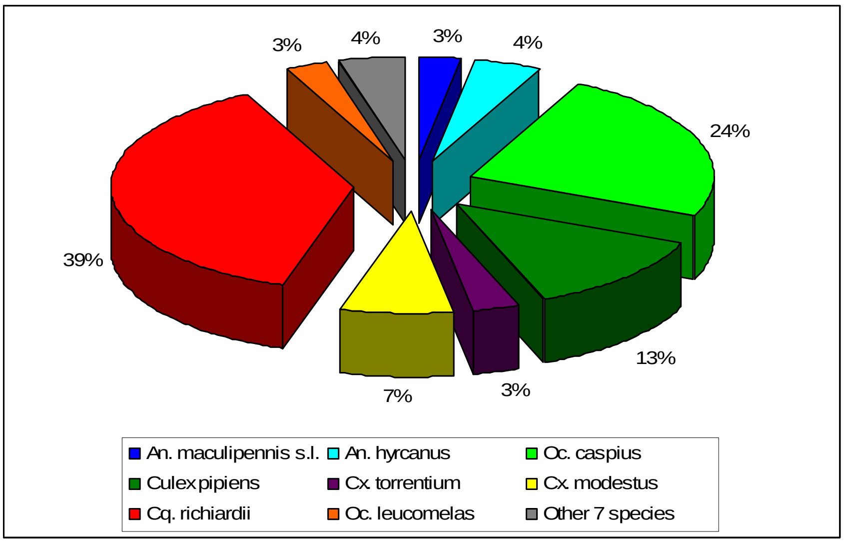
Mosquito species collected in Grindul Lupilor in 2008

The mosquito fauna in Grindul Lupilor has been dominated by the *An. maculipennis s.l.*, *An. hyrcanus*, *Cx pipiens* and *Cx Torrentium*, and in Mila 26 by the same species to which *Cx modestus* is added in a higher proportion than in Grindul Lupilor. *Coquillettidia richiardii* appears in quite low proportions in these 2 sites.
In comparison with last year, *Cx pipiens* appears as dominant species in these 2 areas in the place of *Ochlerotatus caspius* which had a quite low proportion in Grindul Lupilor (6.0%) but not exceeding 0.3% in Mila 26. This species appears in low densities because of the diminishing of its specific breeding sites induced by the drought even in this area. The other 5 species (2.8%) in the image (collected in Grindul Lupilor) were: *Oc. leucomelas, Oc. dorsalis, Oc. zammitii, Uranotaenia unguiculata,* and *Culiseta subochrea*. The other 5 species (1.6%) in the image (collected in Mila 26) were: *Ae. vexans, Oc. leucomelas, Oc. caspius, Uranotaenia unguiculata,* and *Cx martinii*.



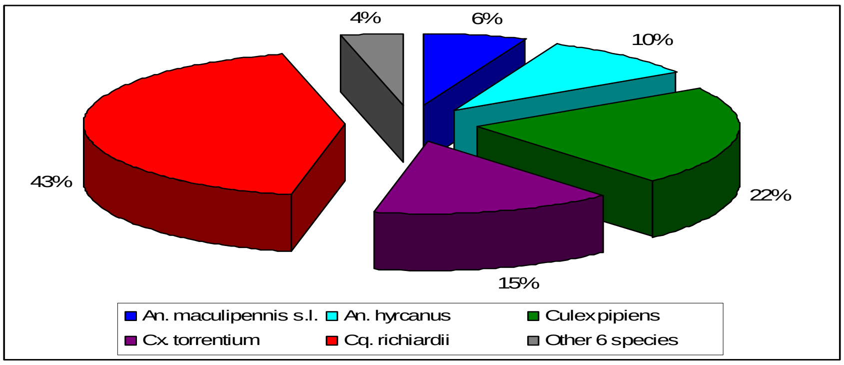
Mosquito species collected in Mila 26 in 2008

Together with *Coquillettidia richiardii* which dominated as usually in highest proportion (39%) the mosquito fauna in Salcioara, *Oc. caspius* has been other dominant species (24%) in the same proportion reached by *Cx pipiens, Cx Torrentium,* and *Cx modestus* together.



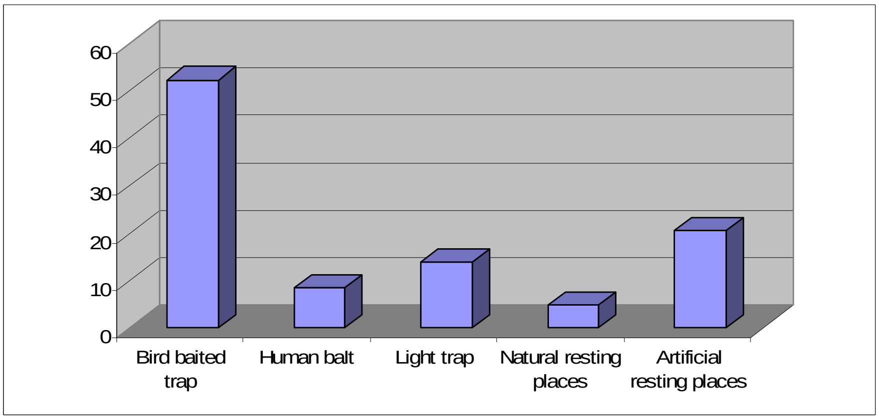
Mosquito species collected in Salcioara in 2008

The other 7 species (1.6%) in the image (collected in Salcioara) were: *Oc. flavescens, Oc. pulcritarsis, Oc. dorsalis, An. Hyrcanus, Cq. Buxtoni, Uranotaenia unguiculata* and *Cx martinii*.



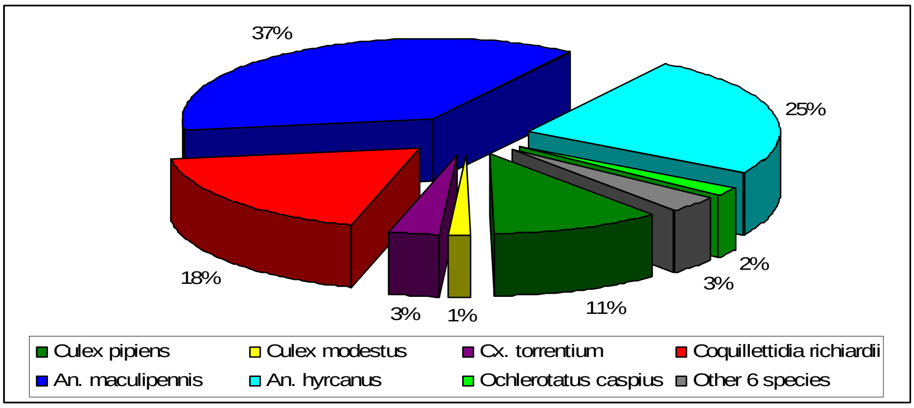
Mosquito species collected in other areas of the Danube Delta in 2008

The investigation of the mosquito fauna in other areas of the Danube Delta (Stipoc Channel and Grind, Raducu, and Rotundu Lakes, Crisan and Ghermandi Channel, Caraorman forest) besides the permanent study areas has been performed in June and at the beginning of October. The species *Cq. Richiardii, Cx pipiens + Cx torrentium* and *An. maculipennis s.l. + An. hyrcanus* dominated the mosquito fauna of these areas in June. The other 6 species (3.8%) in the image were: *Ae. vexans, Cx modestus, Oc. caspius, Culiseta subochrea, Uranotaenia unguiculata* and *Cx martinii*. Comparison between the old and present data regarding the presence and dynamics of mosquito species and correlation with meteorological data are in progress.



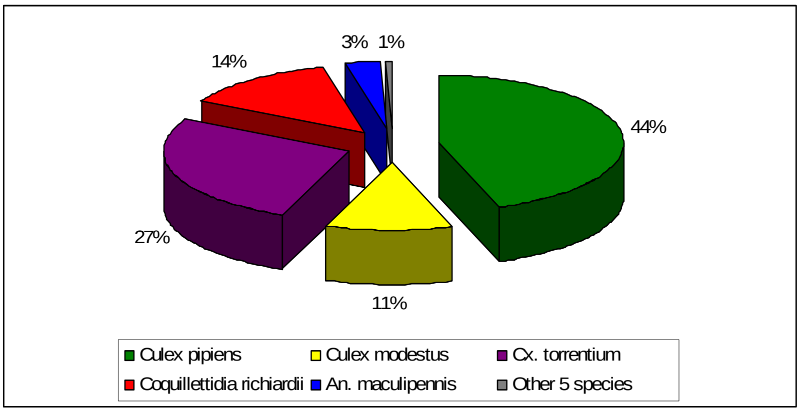
Proportions of mosquitoes collected by different methods (Danube Delta and lagoons) in 2008

A proportion of 79.5% mosquitoes were collected outside by light trap and bird-baited trap, on human bait, and with entomological net from different habitats and types of vegetation and 20.5% by aspirators from artificial resting places.



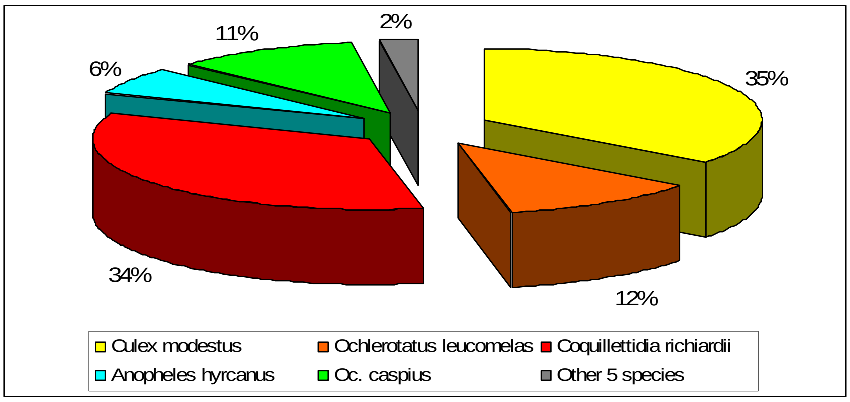
Mosquito species collected by light trap – 2008

The light trap collected as usually the higher number of species from the area (13 species) although only 13.8% from the total number of mosquitoes were collected with this trap. The other 6 species (3.2%) in the image were: *Ae. vexans, Oc. leucomelas, Oc. dorsalis, Oc. pulcritarsis, Cx martini* and *Uranotaenia unguiculata*.



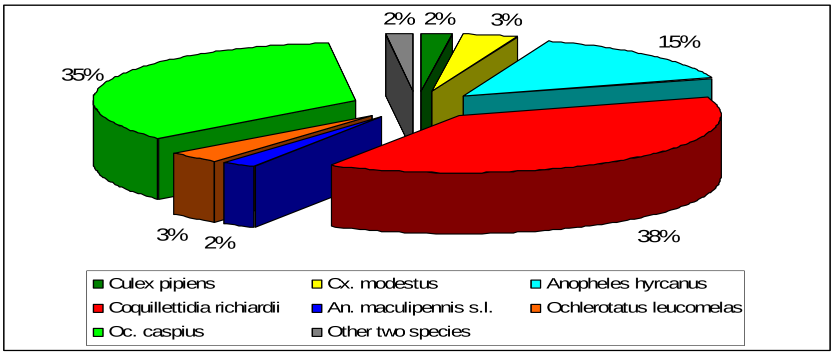
Mosquito species collected by bird baited trap – 2008

Ten mosquito species have been collected with bird-baited traps in this area. They were ornithophilic species which may take the WNV from birds and transmit it to other birds, and other vertebrate hosts. *Culex pipiens* was collected in the highest quantity (idem in 2007) by bird-baited trap: 44.8% of the entire collection was made by this trap (representing 94.4% from all collected specimens of this species). The other 5 species (0.6%) in the image were: *An. Hyrcanus, Oc. leucomelas, Oc. caspius, Cx martinii* and *Culiseta subochrea*.



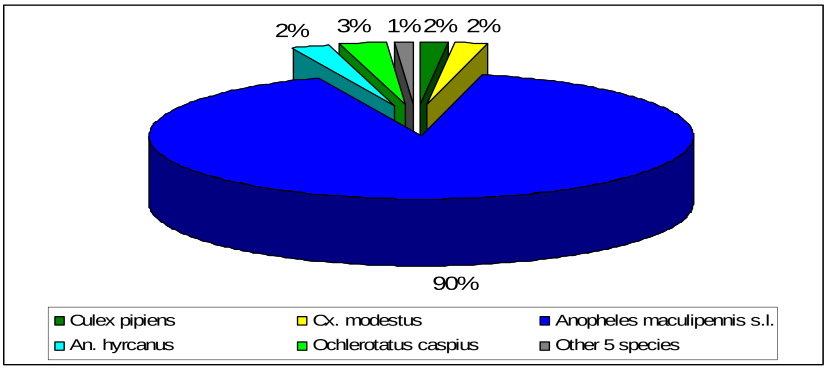
Mosquito species collected on human bait – 2008

The other 5 species (2.4%) in the image were: *Cx torrentium, Ae. vexans, Oc. dorsalis, Oc. flavescens,* and *Cx martinii*. Seven species have been collected both on bird-baited trap and on human bait. This means that the mosquitoes of these species may take the virus from birds and transmit it to humans. The species that seem to be significantly both ornithophilic and anthropophilic are: *Coquillettidia richiardii, Cx modestus, Cx Torrentium, An. Hyrcanus, Oc. caspius. Oc. leucomelas. Cx martinii*.



Mosquito species collected by entomological net in vegetation natural resting places – 2008

Coquillettidia richiardii, Ochlerotatus caspius and *An. hyrcanus* were collected in higher quantity with the entomological net in vegetation. The other 2 species (1.6%) in the image were: *Coquillettidia buxtoni*, and *Ochlerotatus zammitii*.



Mosquito species collected inside the artificial resting places - 2008

Collections inside the artificial resting places (animal shelters, chicken coops, and houses) have been dominated by *An. maculipennis* group as everywhere in the rest of the country. However, other species enter these resting places in higher quantities than they are in other areas of the country. The other 5 species (1.1%) in the image were: *Cx torrentium, Coquillettidia richiardii, Oc. Leucomelas, Culiseta subochrea and Uranotaenia unguiculata.*

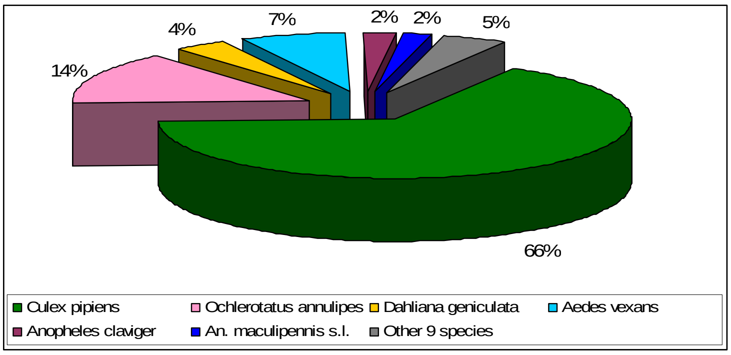
Investigations on the mosquito fauna in Bucharest Metropolitan Area

Investigations on the mosquito fauna in Bucharest metropolitan area have put in evidence extremely low abundance of this fauna until the total lack of mosquitoes in many places and moments this year. The presence in Bucharest urban area without exception of a single species, *Cx pipiens,* because of the climate conditions (as in 2007) has been recorded this year.

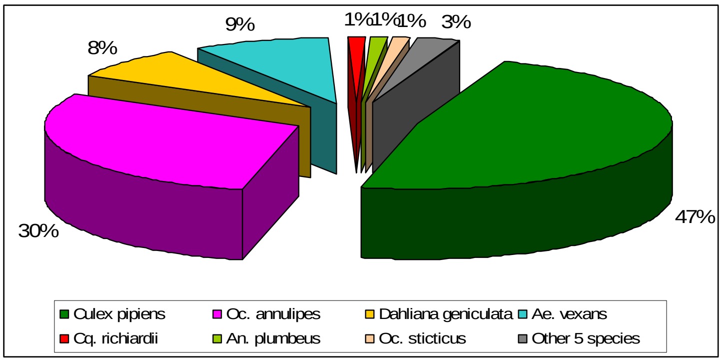
The mosquitoes (a total of 2,921 specimens) have been collected outside on human baits, by bird-baited traps. CDC light traps and gravid traps and inside in blocks and animal shelters in Budeni village. Outside collections in urban Bucharest and 1 Decembrie suburb contained 204 specimens of *Cx pipiens*.

The over-wintering *Cx pipiens* mosquitoes have been collected from blocks in Giurgiu, a town in the Danube valley included in Bucharest metropolitan area (657 specimens). The over-wintering mosquitoes have been missing in blocks in Bucharest in winter of 2007-2008 and in October and November 2008.

The anophelines of *maculipennis* group (1,685 specimens) and 22 *Anopheles claviger* were collected in animal shelters in Budeni village. Outside collections in Comana area (forest, border of Comana lake, courtyard in Budeni village) in May – August 2008 included 375 mosquitoes. They belonged to 15 species (26 species recorded in Romanian Plain where Comana area is located). Although 15 species were found out this year (only 7 species in 2007), the densities of their populations were very low because of the effects of the climate changes in the area in the last years.



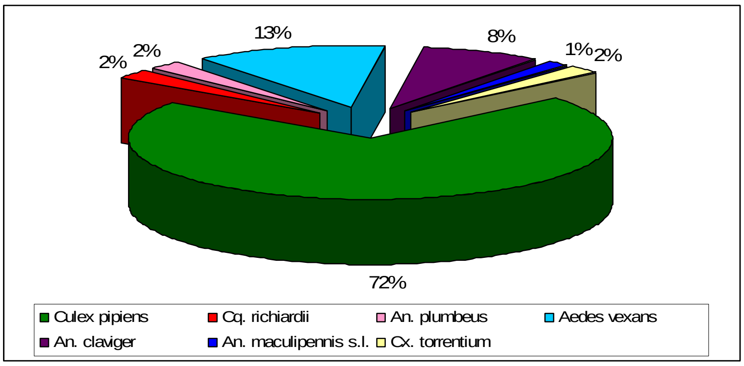
The mosquito species (outside collections) in Comana area – 2008



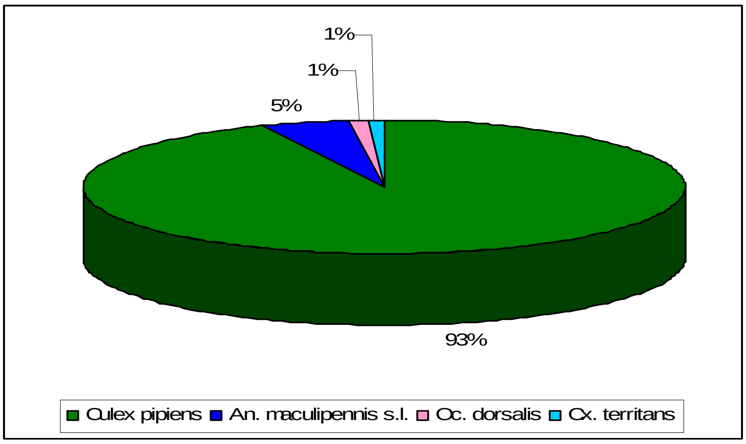
Mosquito species collected in Comana forest – 2008

Among the 12 species collected in the forest, the other 5 species (2.7%) in the image were: *Ochlerotatus cantans, Oc. leucomelas, Oc. caspius. An. claviger* and *An. maculipennis s.l.*

Seven species have been collected at Comana Lake**.** The smallest number of species (4) was collected outside in Budeni village.



Mosquito species collected at Comana lake – 2008



Mosquito species collected outside in Budeni village – 2008

While only one anopheline specimen of *maculipennis* group both in Comana forest and at Comana lake and 5 anophelines outside in Budeni village were collected in 2008, the mean number of anophelines / one animal shelter in Budeni village was about 1,500 – 2,000 specimens.

Investigations for the detection of West Nile virus in mosquitoes

The detection of WNV in mosquitoes has been performed by RAMP system (Rapid Analyte Measurement Platform). Mosquitoes (about 4,200 specimens in 84 pools) were collected from mid May to mid September in the Danube Delta and lagoon area, and in one of Bucharest suburbs (1 Decembrie). Over-wintering mosquitoes were collected in Giurgiu in February and March 2008, in Tulcea in November 2007 and 2008 and in Grindul Lupilor in November 2008. Three pools of *Cx pipiens* + *Cx torrentium* were collected in June 2008 on the Channel and Grind Stipoc, one pool of *Cx pipiens* and one of *An. maculipennis s.l.* were collected in September 2008. In Mila 26, three pools of over-wintering *Cx pipiens* were collected in Tulcea in November 2007, and four pools in 2008. Two pools of *Cx pipiens* collected in November 2008 in Grindul Lupilor (one containing females and the other males) were positive for WNV.

Detection of West Nile virus in mosquitoes by RAMP test (Romania – 2008) (positive samples)

Locality	**Mosquito species**	**Date of collection**	**No. of mosquitoes**
Tulcea	*Cx pipiens*	November 2007	56 ♀
Tulcea	*Cx pipiens*	November 2007	57 ♀
Tulcea	*Cx pipiens*	November 2007	46 ♀
Channel (1 km to Stipoc)	*Cx pipiens + Cx torrentium*	June 2008	52 ♀
Idem	*Cx pipiens + Cx Torrentium*	June 2008	50 ♀
Grind Stipoc	*Culex pipiens + Cx torrentium*	June 2008	75 ♀
Mila 26	*Cx pipiens*	September 2008	60 ♀
Mila 26	*An. maculipennis s.l.*	September 2008	43 ♀
Tulcea - garage	*Cx pipiens*	November 2008	4 pools (300 ♀)
Grindul Lupilor	*Cx pipiens*	November 2008	60 ♀
Idem	*Cx pipiens*	November 2008	80 ♂

The very significant results are the detection of WNV in:

- over-wintering mosquitoes (*Cx pipiens*);
- a pool of *An. maculipennis s.l.* females; anophelines of *maculipennis* group have a very extended distribution in high densities over all the former malaria endemic areas;
- a pool of males of *Culex pipiens,* which could demonstrate the trans-ovarian transmission of WNV by this species.

All these facts, and the appearance of human WNV neurological infection cases some years ago in April and early May, confirm the endemic status of WNV in Romania.

The viral strains isolated from mosquitoes by us in 2002 and 2007 will be molecularly characterized using a multi-level protocol which puts in evidence and identifies WNV in steps: RT-PCR, nested PCR, and sequencing. The protocol includes also the new Lab-on-a-chip technology for checking the quality and quantity of total viral RNA. The multi-level protocol is established in our laboratory. The work will be performed in the new molecular unit of the new location of our laboratory in the Institute.

FRANCE: EID

Vector-Host contact rate

Considering the high vector-host contact in our bird-baited trapping results during the first months of the year, we conducted a special trapping protocol to collect information on the resumption of biting activity of *Culex* mosquitoes. We trapped mosquitoes with birds 2 times a week, almost every week from February to April. We worked in 2 different areas: the first one is the stone quarry of Sussargues where we previously found a very high peak of *Cx pipiens* collected on birds in March. The second one is the ornithological reserve of Pont de Grau, where we have accurate data on host species and abundance. We collaborated with the French ornithologist team to have data on host availability in both trapping areas. Few mosquitoes were, probably because of the unfavourable meteorological conditions. However, we should have enough data to study the influence of host availability on biting rate, an important parameter to evaluate the risk of WNV circulation.

Study of the two supposed forms of *Culex pipiens*: *pipiens* and *molestus* biotypes

We tried to distinguish urban from rural forms of *Cx pipiens*. We collected larvae from different breeding sites and brought them back in the laboratory to study their stenogamy and autogeny, two characteristic of this form. We also studied *Cx pipiens* mating behaviour from:

- *urban areas*: Montpellier, Arles, Port Saint Louis, Perpignan and Nice sewers
- *suburban areas*: Teyran and Pérol wastewater treatment plants, Méjean pond.
- *rural areas*: Méjanes and Charnier ponds.

All these mosquitoes — except a few females from Méjean pond — were eurygamous. The two different supposed biotypes are not clearly differentiated between urban and rural areas, as it is in London, New York, or in South Africa. We assume that *Cx pipiens* can be considered as a homogenous vector between his urban and rural forms, in the spatial treatment of the risk. The presumed biotypes do not seem to be responsible of the mostly suburban and rural past circulations of WNV in France. A population genetics study is planned for confirmation, using microsatellites.

Genetic study of *Cx pipiens* populations (collaboration with C. Toty, IRD-LIN)

The goal of this study is to define if there are differences in *Culex pipiens* genetic signature in southern France, and if we could link them to different behaviour or biotopes. After a bibliographical study, we selected 11 microsatellites used in different publications from different countries for characterization of different forms of *Culex pipiens: pipiens, molestus* and *quinquefasciatus*. We selected 27 different samples of mosquitoes collected in 2006, from the early beginning to the end of the seasons, and from different study areas. They were trapped with different methods (mammals, birds or carbon dioxide). We also added mosquitoes from different cities of the French Mediterranean coast (Perpignan, Montpellier, Arles, and Nice). This work is in progress. For the time being, we tested 6 samples - as different as possible - with 4 primers. Some observed differences may be more than a simple polymorphism, but for the moment, the number of tested mosquitoes is too low to draw conclusions.

Other data:

We can use a collection of mosquitoes trapped regularly in the Camargue by the EID in 2008 for seasonal dynamic analysis, modelling or additional genetic studies (depending on later conclusions).

Perspectives:

Some environmental data seem to be similar during the previous circulations of WNV in France, and seem to fit with our entomological results. We have to confront these preliminary results with the ornithological data of the French team, during a forthcoming meeting. We shall then examine whether this hypothesis is relevant.

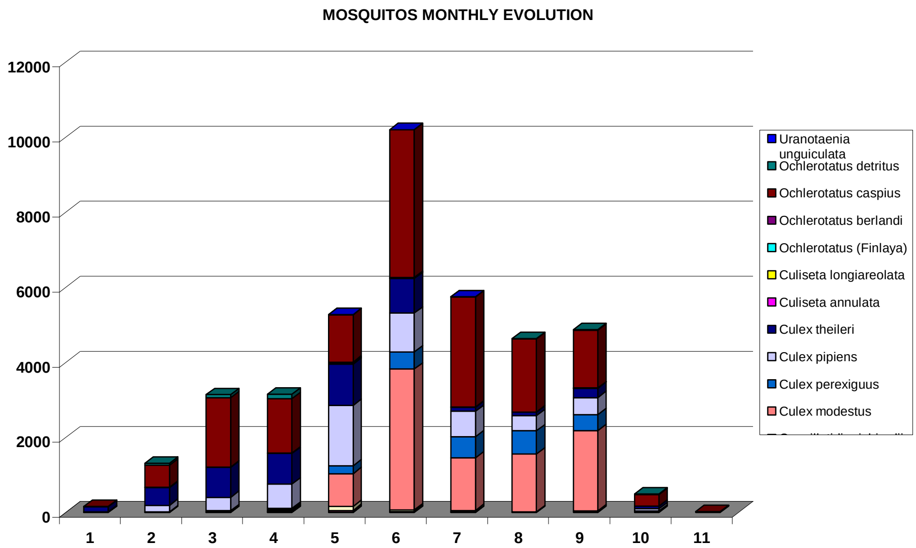
CZECH REPUBLIC: IVB

Mosquitoes were collected at 2-wk intervals at the two WNV study sites (Nesyt fishpond - 1 location; game preserve Soutok - 2 locations) between 15 April and 31 October 2008, using pigeon-baited traps and CO_2-light CDC traps, situated in pairs one at 1-1.5 m. the other in the canopy at 5-6 m above ground), and exposed for 2 nights.

A total of 6,781 mosquitoes of 19 spp. were captured (Nesyt + Soutok. resp.): *Culex pipiens* (589+174). *Cx modestus* (136+2), *Aedes vexans* (389+4775), *Ae. cinereus* (84+0), *Ae. rossicus* (0+5), *Oc. cantans* (355+12), *Oc. flavescens* (9+0), *Oc. excrucians* (0+4), *Oc. cataphylla* (7+8), *Oc. caspius* (1+0), *Oc. sticticus* (18+318), *Culiseta annulata* (105+3), *Cs. morsitans* (2+0), *Coquillettidia richiardii* (29+4), *Uranotaenia unguiculata* (5+0), *Anopheles maculipennis* s.l. (42+27), *An. plumbeus* (1+43), *An. claviger* (50+3), *An. hyrcanus (pseudopictus)* (57+0) – the latter species being a new record for Czechland.
In comparable pairwise trials, 356 mosquitoes (340 *Cx pipiens*) were caught in bird-baited traps, whereas 6,425 mosquitoes in CO_2-light traps. A total of 171 mosquitoes were caught in the ground bird-baited traps while 185 mosquitoes in the canopy bird-baited traps; analogical figures for the CO_2-light traps were 6,079 and 346, respectively, and nearly exclusively *Cx pipiens* was found in the canopy traps.
Virological examination of the mosquitoes collected (and stored at -60 ºC) in the years 2006 (the rest), 2007, and 2008 by both the i.c. inoculation of suckling mice and on Vero cell tube cultures involved 201/8,392 mosquitoes (no. pools/no. individuals): 48/1,937 *Cx pipiens,* 8/219 *Cx modestus,* 2/60 *Cs. Annulata,* 92/4572 *Ae. Vexans,* 9/396 *Ae. Cinereus,* 8/246 *Ae. Rossicus,* 17/651 *Oc. Cantans,* 2/28 *Oc. Caspius,* 2/21 *Oc. Sticticus,* 2/73 *Oc. Cataphylla,* 1/27 *Cq. Richiardii*, 6/90 *An. maculipennis* s.l., 4/45 *An. plumbeus*, 1/48 *An. Claviger*, and 1/49 *An. hyrcanus*; of these mosquitoes, 7/376 were overwintering *Cx pipiens*.
No sample yielded a virus. However, RNA of WNV was detected by PCR in one pool of 50 female *Cx pipiens* out of 3,013 mosquitoes (58 pools) of this species overwintering in cellars in South Moravia, 2001 through 2008.

SPAIN: EBD

We have sampled mosquitos every 2 weeks. A total of 39,145 mosquitos had been sampled. Very low captured rates were observed during the winter. According to the year, January and December are the month with minimum captures. *Culex pipiens, Cx modestus, Cx theileri and O. caspius* are the dominant species.



A set of 491 pools of mosquitoes were tested for Flavivirus, 38.9% were positive. Ten species were tested: *Cx pipiens (*52.6%), *Cx theileri* (52.1%), *Cx modestus* (33.3%), *Culiseta annulata* (16.7%), *Cx perexiguus* (34.8%), *Culiseta longiareolata* (25.0%), *Ochlerotatus caspius* (33.8%), *Ochlerotatus detritus* (17.4%), *Anopheles atroparvus* (25.0%). *Anopheles algeriensis* (6 pools) and *Uranotaenia unguiculata* (1 pool) were negatives.

ROMANIA. DANUBE DELTA: DDNI

This overview was submitted by the DDNI team (ornithology, WP4) who has worked in tandem with the NIRDMIC PhD entomologist. It gives information additional to that included above under NIRDMIC:

Characterization of the mosquito vector community in the Danube Delta Biosphere Reserve and Dobrudja Tableland

In the last report, we achieved the characterization of the diversity of reservoir (birds) associations in space and time (bird database and GIS bird habitat map). During this reporting period, we carried on mosquito investigation for all bird habitats. As for birds, we must characterize the diversity of vector (mosquitoes) associations in space (Danube Delta Biosphere Reserve and Dobrudja Tableland) and time.

Besides supporting of the Cantacuzino PhD student for mosquitoes collection in the study areas, we participated at the mosquito collection outside of the study areas. We studied mosquito habitats to assess whether they are distinct, like for birds. We investigated mosquito fauna twice, in 10 bird habitats (Figures 2 – 5), outside of the study areas, in 2008. In summer we used a boat equipped with an engine (accommodation at DDBRA ranges). In autumn we traveled with a large ship (accompanied by the same boat, both belonging to DDNI – Fig. 6), used also for accommodation. To avoid redundancy, the detailed data will be available on Cantacuzino Institute Report. Adding the investigated habitats within the study areas, we characterized about half of the total number (35) of bird habitats. In 2009, the other half of the bird habitats will be investigated. Because the EDEN project will stop in early 2010, all data must be integrated into a georeferenced database at the end of spring and beginning of summer 2009.

The design of mosquito database is similar with the one made by entomologists, ornithologists, and teledectionists from CIRAD and EID – Montpellier. It has to be adapted to the habitats and conditions of Danube Delta Biosphere Reserve and Dobrudja Tableland. Our colleagues from Cantacuzino Institute will use for database integration, as a background, the DDBR Vegetation Map and Bird Habitat Map, both in Arc View format. As soon as we obtain (from CIRAD) the method for characterizing the diversity of horse reservoir associations in space, we will start to work on it.

Fig. 2-5. Mosquito investigation in the bird habitats: (at the edge of the) clair de roselier, natural floodplain forest, forest (mixed oak wood on marine levee), and riparian forest

Fig. 6. The ship and the boat equipped with an engine used for mosquito investigations

Mosquito investigations in the Danube Delta Biosphere Reserve

There were found positive to WNV in 2007 two pools of *Coquillettidia richiardii* (one from Maliuc – Mila 26 and one from Sălcioara) and two pools of *Culex pipiens*, both of them at Maliuc – Mila 26. Also, some positive pools were found from mosquitoes collected in Tulcea: a garage and a cellar where we carried out the assessment of the winter survival rates of mosquitoes.

Again, in 2008, we have supported the working conditions, licenses and accommodation for Liviu Prioteasa, the Cantacuzino Institute's WNV PhD Student, responsible for mosquito collection in the Danube Delta. To avoid redundancy, data will be available in Cantacuzino Institute's report. We will only outline some characteristics of mosquito fauna in DDBR on 2008.

As in 2007, the mosquitoes were collected in 3 study areas: Grindul Lupilor, Sălcioara, and Maliuc – Mila 26. We found an increasing number of species: 18, vs. 12 in 2007. The mosquito fauna in DDBR has been dominated in 2008 by *Cx pipiens, Ochlerotatus caspius, Anopheles hyrcanus* and mosquitoes from the *maculipennis* group, the identified species being *An. atroparvus* and *An. messeae*.

(b) Overwintering. The study by the NIRDMI PhD entomologist and the DDNI ornithologists will continue in the winters of 2007-8 and 2008-9. It should be possible for other entomology teams to locate overwintering sites and follow a similar protocol. Whenever possible, sites should be representative of ecotopes identified in WP1.

Estimation of the survival rate of overwintering mosquitoes

Winter survival rates of mosquitoes and WN minimum infection rates (MIR) will be key parameters in models of epizootic transmission. To test over-wintering mosquitoes and assess the survival rates of over-wintering *Cx pipiens*, we found a structure in 2007 (in Tulcea – a cellar and a garage) with a high number of over-wintering mosquitoes. Last year were tested a few hundreds mosquitoes, and some pools were positive for WNV. At the beginning of the winter 2007-08, we marked with fluorine 211 mosquitoes, and we assessed their survival rate (Fig. 1 and 2). At the beginning of the spring 2008, it was about 2%. We have repeated the experiment for the winter of 2008-09. We captured 300 mosquitoes from the same cellar and garage in Tulcea in November of 2008, all of them being released in a special mosquitoes cage (Fig. 3 and 4). The mosquitoes were not marked to avoid a possible effect of fluorine on mosquito survival. A large white paper was laid on the floor of the cage, enabling the monthly count of dead mosquitoes.

Fig. 1 and 2. Marking mosquitoes with fluorine to assess *Cx pipiens* survival rate during winter 2007-08

Fig. 3 and 4. Mosquito special cage - Tulcea

Because last year we found some pools positive to WNV, we decided to collect other mosquitoes in November and in December 2008. In November, several pools of newly emerged *Cx pipiens* from a septic tank tested positive for WNV by RAMP test, including one pool of males (indicating vertical transmission). For this reason, the site was revisited to make an intensive collection of larvae, pupae and adults, which were returned to the laboratory in Bucharest for rearing and testing for virus.

(c) Role of ectoparasites. Collection of ectoparasites during bird ringing/bleeding is labour intensive and as not proved productive; to date, only the DDNI team have come up with significant collections, although the IZSAM team has collected some ticks. None of the Danube Delta *Mallophaga* has tested positive, although two were from seropositive birds. While this data is sparse, collection should continue; further negative results from seropositive birds will be significant information. A search of nests during ringing of juveniles (e.g. storks) is encouraged.

ITALY: IZSAM and ISS

All ticks identified in 2007 were negative for both virus isolation and PCR.

Table 5. Ticks found on birds in 2007

Date	Bird	Tick	♀	Nymphs	Larvae	PCR	Virus isolation
11/05/07	*Acrocephalus scirpaceus*	*Ixodes ricinus*	-	1	-	Neg.	Neg.
11/05/07	*Acrocephalus scirpaceus*	*Hyalomma aegyptium*	1	-	-	Neg.	Neg.
23/05/07	*Turdus philomelos*	*Scaphixodes frontalis*	1	-	-	Neg.	Neg.
09/10/07	*Turdus merula*	*Ixodes ricinus*	-	3	-	Neg.	Neg.
09/10/07	*Phylloscopus collybita*	*Pholeoixodes arboricola*	-	-	1	Neg.	Neg.

ROMANIA. DANUBE DELTA: DDNI

In 2007, 38 samples with ectoparasites were harvested from 37 birds. In November of 2007, 24 samples of *Mallophaga* were tested with RAMP, all of them being tested together. The result was negative for WNV even though two samples of parasites were taken from two positive birds.

Results in 2008: Collections of ectoparasites on resident and migratory wild bird species: Despite of our higher efforts, we gathered only 11 samples: *Mallophaga* – 9 samples from 5 species of birds; and *Ixodidae* – 2 samples from 2 species of birds, table 1 and 2. We suspect that the amount of ectoparasites collected in 2007 was not big enough and RAMP test was negative. The amount of the collected lice in 2008 is much smaller than the tested lice in 2007 (9 samples in 2008, 24 samples in 2007). For this reason, we have decided to not test the lice with the RAMP test. All ectoparasites (lice and ticks) will be sent for WNV testing to IZS, Italy, in November or December 2008.

Tab. 1. Bird host species and the ectoparasites (*Mallophaga* and *Ixodidae*) collected in 2008.

Crt. no.	Bird host species	Date	Location	Ectoparasites
1	*Corvus frugilegus juv.*	11.02	Murighiol	*Mallophaga*
2	*Emberiza schoeniclus M*	08.04	Grindul Lupilor	*Mallophaga*
3	*Emberiza schoeniclus F*	08.04	Grindul Lupilor	*Mallophaga*
4	*Sturnus vulgaris*	09.04	Grindul Lupilor	*Mallophaga*
5	*Sturnus vulgaris*	28.05	Grindul Lupilor	*Acari* (*Ixodidae*)
6	*Sturnus vulgaris*	29.05	Grindul Lupilor	*Mallophaga*
7	*Sturnus vulgaris*	30.05	Grindul Lupilor	*Mallophaga*
8	*Lannius collurio juv.*	20.08	Grindul Lupilor	*Mallophaga*
9	*Turdus merula M*	28.10	Grindul Lupilor	*Mallophaga*
10	*Turdus merula M*	29.10	Grindul Lupilor	*Acari* (*Ixodidae*)
11	*Turdus merula M*	29.10	Grindul Lupilor	*Mallophaga*

Tab. 2. Lice (*Mallophaga*) species collected in 2008 and their bird host species

Crt. no.	Bird host species	Lice (*Mallophaga*) species
1	*Corvus frugilegus (L.)*	*Brueelia tasniemae (Ansari)*
		Colpocephalum frugilegi (Zlotorzycka)
		Philopterus atratus (Nitzsch)
2	*Sturnus vulgaris (L.)*	*Brueelia nebulosa (Burmeister)*
		Myrsidea cucullaris (Nitzsch)
3	*Lanius collurio L.*	*Menacanthus canelinus*
4	*Turdus merula L.*	*Brueelia merulensis (Denny)*
		Docophorulus (Philopterus) merulae (Denny)
5	*Emberiza schoeniclus L.*	*Docophorulus residuus (Zlotorzycka)*

ITALY: IZSAM and ISS

No data for overwintering species

(d) Horse-biting species. None of the participants has approached this WP2 topic. It remains important to know which ornnithophilic species are biting horses (and, as far as possible, which bite humans). Simple aspirator collections around an animal, in suitable places, are all that is required. We can assume biting in the evening crepuscular period. A few collections during each of the intensive trapping periods would fill this gap. Collections will be particularly important in the season-late summer to autumn-when equine cases have been most numerous. The observation in the Danube Delta that some populations of *Cx modestus* appear more mammalophilic than others is extremely interesting, and should be pursued where possible.

France, EID: study of *Cx pipiens* and *Cx modestus* hosts' preferences

Because we cannot breed eurygamous larvae in the laboratory, we worked with *Culex* females trapped on the field. We offered them the possibility to feed on a bird or a guinea pig. Two methods were used:

- in the laboratory, under controlled conditions of temperature and humidity,
- on the field, under natural conditions.

Almost 20 tests were conducted during the last summer. *Culex* mosquitoes were trapped from different places (Arles, Méjanes, and Charnier pond in the Camargue), with carbon-dioxide and bird-baited traps. Only a few females have bitten the hosts in this experiment (1 bite / 100 mosquitoes). Collections with carbon dioxide provided thousand of *Culex* and gave stronger results. All the collected *Cx pipiens* were preferentially ornithophilic, but also fed on mammals. *Culex modestus* tended to feed both on birds and mammals. Our complete results are still under statistical analysis.

Deliverables

D WNV-04 Seasonal profiles of adult mosquitoes at 1-2 week intervals (M28 – M36). Excellent progress; all teams have good databases, and most at 2-week intervals

D WNV-05 Seasonal profiles of ornithophilic and horse-biting activity at 1-2 week intervals. coupled with a daily record of climate variables (M28 – M36). Again, excellent efforts, though data on horse-biting activity not available in some study areas (few horses).

D WNV-06 Tests for WNV in collections of ectoparasites associated with birds—resident and migratory—captured for serosamples (M28 – M36). Teams involved have made efforts (the job of collecting ectoparasites is finicky and it appears that many birds have relatively few of them).

.D WNV-07 PCR results for WNV in bird ectoparasites for sero-negative and seropositive birds (M28 – M36). As for WNV-06

D WNV-08 PCR or VecTecresults for WNV in over-wintering *Culex pipiens* populations (M26 – M28. M36 – M40). An alternative apparatus, the RAMP, was selelected instead of VecTec (published evaluations in the USA reported much higher accuracy). Results are fascinating and exciting, but await confirmation by virus isolation.

D WNV-09 Survival rates of over-wintering Cx pipiens (M26 – M28. M36 – M40). The NIRDMIC student, Liviu Florian, in collaboration with DDNI, put effort into this study, with limited but interesting results. He is repeating this in the winter of 2008-09.

Milestones and expected result

M WNV5 - Seasonal profile of adult mosquitoes, biting activity and host preference in study areas (M36). The data over the past years has varied according to climatic variation; seasonal patterns have been markedly different in different regions, and species profile has varied accordingly. The contrasts between regions will be particularly interesting when the data for different regions are brought together.

M WNV6 - Abundance of adult mosquitoes in the context of climatic variables (M36). As with WNV5, abundance data can be compared between regions

M WNV7 - Evidence for/against role of ectoparasites in WNV transmission (M36). Despite energetic efforts, the numbers of ectoparasites collected have been far lower than expected. A number of samples remain to be tested for virus, but the low quantity of data will be too low for meaningful conclusion. Having said this,

even a single positive pool would be an important piece of information M WNV8 - If there is evidence of current WNV transmission (seroconversion in birds, or horses, or clinical cases in horses or humans), pools of mosquitoes will be tested for virus (M36). Several pools of mosquitoes tested with the RAMP kit have proved positive. Confirmation by isolation from the same samples (pools were split) will come later. M WNV9 - Winter survival rates of mosquitoes and WN minimum infection rates (MIR) will be key parameters in models of epizootic transmission (M30). Studies in the DDNI region revealed a high survival rate and positive mosquitoes. If confirmed, this will be an important finding.

WP WNV 3 – Public health and human activities

Work package number	WNV3	Start date or starting event:			37
Participant id	NIRDMI	IVB			Total
Person-months / participant	2	2			4
Objectives A serosurvey of the human population is highly desirable, but requires complex legal procedures and an immense amount of effort. Nevertheless, it is clear that seroprevalence is a critical parameter for assessment of the true public health significance of the virus.					

Work performed during previous reporting periods

The review of historical information on incidence/prevalence of WNV in humans and horses has been very instructive. Excellent data are now available for most of the teams. Seroprevalence in horses are continuing under WP WN 4. A serosurvey of the human population is much more difficult, however, because it requires complex legal procedures and an immense amount of effort.

Description of work

The two teams who were interested to pursue this (IVB and NIRDMI) have not made much progress: legal/ethical considerations, particularly under new EU rules, make this a daunting task. They are encouraged to use their judgment as to whether to continue, but we probably will have to drop this objective. In the event of a significant human outbreak, a serosurvey would probably be easier to organize.

Achieved results:
ROMANIA. BUCHAREST: NIRDMIC
Human cases

The special climate conditions in Romania in the last 3 – 4 years influenced the mosquito fauna. It had an extremely low abundance in the southern half of the country, as well as in the rest of the country. Even in the Danube Delta and lagoon area, the size of mosquito populations has been much lower than in other years.
One case of WNV human neurological infection (meningitis) was reported in the Romanian Plain not far from the Danube, and 2 cases of WN febrile respiratory disorders accompanied with rash were reported in Sibiu district, Transylvania Province. This very low number of WNV human cases is in correlation with the very low abundance of mosquito vectors, or even their absence in many places, during the epidemic season. Year 2008 is the fourth year after the outbreak in 1996 with the smallest number of cases in Romania. The two years with only 2 cases each were 2006 and 2007, when the unusual climatic factors induced very low abundance of the mosquito populations. This situation continued in 2008. A small number of cases (only 3) were also registered in 2002, a very droughty year with very low mosquito populations.
Human cases were reported every year after the outbreak in 1996, the total number being 95 cases until now with a general level of lethality of 6.3% (the mortality of the same level was registered in the outbreak too). The cases in 1997 – 2008 appeared on a very large territory including Romanian Plain and Dobrogea, but several cases were registered in the middle of Transylvania and in Moldova counties.

The analysis of the monthly values of precipitations and temperatures (mean, min and max) has been performed for the areas where previous WNV human outbreaks have been registered in Romania (Târgu Mureş – 1955, Oraviţa – 1965, Bucharest metropolitan area – 1996). The analysed values of precipitations and temperatures were from one year before outbreak and the year of outbreak. A period with higher temperatures and precipitations than the mean reference values (per about 60 years) of every area was registered before every WNV outbreak. This result seems to show that the higher temperatures and precipitations before WNV outbreaks have induced the increase of the mosquito vector populations registered in all this situations.

CZECH REPUBLIC: IVB

No human case of West Nile fever was reported in Czechland during the period.

SPAIN: EBD

Neither outbreaks nor individual cases of confirmed or suspected WNV infection are available in public health and veterinary historical records.

Deliverables D WNV-17: An estimate of flavivirus seroprevalence in the human population (By M48). Serosurvey had had to be abandoned.
Milestones and expected result M WNV19 - An assessment of risk of infection in humans in areas where transmission is known to occur (M48). The incidence of human cases indicates a low transmission rate, or at least a low frequency of clinical cases, even in the region in Italy where there were many equine cases (see WNV-4)

WP WNV 4 – Animal reservoirs

Work package number	WNV4	Start date or starting event:					37
Participant id	DDNI	IVB	CNM	IZS	CIRAD	UNISAP	EBD	NIRD MI
Total per participant	19	31	10	19	20	6	14	19
Participant id	IPP							Total
Total per participant	24							162

Objectives

The general objective of this WP is to clarify the role of domestic and wild animals in the epidemiology of WNV in Europe by quantifying WNV prevalence in sensitive animals (horses) and testing migrant and resident reservoirs (birds). During the next 18 months, the specific objectives are to:

- determine seroprevalence of WN virus in resident and migratory birds, and in horses
- identify bird species with highest infection rate
- monitor infection rate by seroconversion in juvenile birds, recaptured birds, and repeat sampling from horses

Rationale for a third season has already been set out under WP1. The progress of the CIRAD team will warrant modification of their trapping objectives, based on completion of serological tests by IP.

Work performed during previous reporting periods

(a) Seroprevalences in birds:

Trapping of birds was conducted in France, Romania, Spain, and Czech Republic and Italy. A sampling strategy was developed to maximize the number of samples, optimize the range of habitats, and optimize comparative studies; a limited number of representative species was selected. Significant number of birds were captured and sampled for serology. Problems in France with diagnostics based on ELISA screening summarized in Section 2. Field studies were conducted in Spain, France, Italy, Czech Republic and Romania. Results indicated a very limited WN transmission, except in Romania.

Additional information: i) some teams had problems in getting the necessary permits for access and work in protected area and on protected species. ii) The outbreak of H5N1 influenza virus in many countries, activities were made more difficult and for example in Romania, bird sampling has been put on hold.

(b) Seroprevalence in horses

Seroprevalence surveys in Romania indicate a high transmission rate in the region adjacent to the Danube Delta. Significant transmission in the Bucharest area is also clear. In Italy, the results also indicate the circulation of the virus. Field studies were conducted in Spain, France, Italy, Czech Republic and Romania. Results indicated a very limited WN transmission, except in Romania.

Description of work

The techniques used in previous years will remain unchanged. Participants in each region will determine the target species for intensive study. Comparison of results to date during the Annual meeting in January 2008 will be important in determining these species.

Achieved results:

ITALY: IZSAM and ISS

As previously, IZS stipulated a contract with an ornithologist and a protocol was arranged to perform all activities on birds (trapping. collection of ectoparasites and blood samples), from 27 February 2006.
Trapping of birds started from 16 April and lasted until the 16 September. Trapping was performed fortnightly as planned in the trapping protocol. Bird trapping was performed using mist nets located in "Le Morette"; 48m of mist nets were located on a North-Southern direction (lat. 43.80750 and long. 10.81481) while another 60m were located on a East-Western direction (lat. 43.80712 and long. 10.81363) for a total length of 108m. Mist nets were located along water canals inside the Fucecchio marshes. Therefore, trapping was biased favoring migratory and resident birds preferring this range of ecotopes. The total trapping was

performed over 138 hours split in 10 trapping shifts. Trapping effort is reported in Table 6.
Every shift lasted two consecutive days, from 4 or 5 pm to sunset of the first day and from one-hour past dawn to 13 am of the second day (mist nets were left open overnight). Birds were collected every hour during trapping shifts. This particular shift was used to avoid heat-exhaustion mortality in birds due to hot weather conditions. The first and the last trapping shift have been performed on a daily bases from dawn to one-hour past sunset. Birds with a body weigh less than 9 g were not bled as the resulting serum quantity would have been insufficient for further serologic testing.
The volume of blood collected from birds was the maximum when compared to the body weigh (1.6% of body weight). Recaptured birds were re-bled only when a period of at least 15-20 days elapsed between the two trappings; this protocol was adopted to allow birds to reconstitute their blood volume before bleeding again in order to avoid shock syndrome. Serum samples were kept at 0-4°C during transit to the laboratory were a serum neutralization test (SN) was performed on every sample.
A capture index was calculated for species captured more frequently, to figure out the presence and abundance during the whole trapping period. The capture index was not calculated for swallows as this species was captured using decoy that would have biased the index. An ornithological database was created and sent to GIS Database Manager Environment Research Group Oxford.

Table 6. Bird-trapping (from 16/04/2008 to 16/09/2008)

Species	Birds	Blood samples	Recaptured birds	Double bleedings	Sera not processed	Positive samples	Negative samples
Lanius collurio	1	1	-	-	1		-
Delichon urbica	2	1	-	-	-		1
Gallinago gallinago	6	4	-	-	2	-	2
Sylvia borin	1	1	-	-	-		1
Cisticola juncidis	2	-	-	-		-	
Amandava amandava	313	-	29	-		-	
Hippolais icterina	1	1	-	1		-	
Acrocephalus scirpaceus	160	113	40	7	25	-	88
Acrocephalus arundinaceus	26	24	5	1	4	1	19
Sylvia atricapilla	10	9	-	-	1	-	8
Carduelis carduelis	8	7	-	-	2	-	5
Aegithalos caudatus	26	-	8	-		-	
Charadrius dubius	3	2	-	-	2		-
Cuculus canorus	2	2	-	-	-		2
Acrocephalus schoenobaenus	5	5	-	-	1	-	4
Acrocephalus melanopogon	3	3	1	-	-		3
Gallinula chloropus	2	2	-	-	1	-	1
Merops apiaster	1	1	-	-	1		-
Asio otus	1	1	-	-	-	-	1
Phylloscopus trochilus	2	1	-	-	-	-	1
Phylloscopus collybita	1	-	-	-	-		-
Alcedo atthis	55	47	4	2	17	-	30
Turdus merula	3	3	-	-	-		3
Tringa nebularia	1	1	-	-	1		-
Passer domesticus italiae	4	3	-	-	-		3
Passer montanus	35	31	4	-	21	-	10
Luscinia svecica	2	2	-	-	-	-	2
Erithacus rubecula	1	1	-	-	-	-	1
Hirundo rustica	81	70	-	-	13	-	57
Locustella luscinioides	3	3	1	1	-	-	3
Saxicola torquatus	4	4	1	-	3	-	1

Species	Birds	Blood samples	Recaptured birds	Double bleedings	Sera not processed	Positive samples	Negative samples
Saxicola rubetra	1	1	-	-	-	-	1
Sturnus vulgaris	1	1	-	-	-	-	1
Ixobrychus minutus	1	1	-	-	-	-	1
Riparia riparia	1	1	-	-	-	-	1
Luscinia megarhynchos	7	6	2	-	2	-	4
Cettia cetti	68	46	21	6	15	-	31
Carduelis chloris	2	2	-	-	-	-	2
Serinus serinus	1	-	-	-	-		-
Grand total	**847**	**401**	**116**	**17**	**113**	**1**	**287**
% of birds	**100.0%**	**47.3%**	**13.7%**	**2.0%**	**13.3%**	**0.1%**	**33.9%**
% of samples	**-**	**100.0%**	**-**	**4.2%**	**28.2%**	**0.2%**	**71.6%**

ROMANIA. BUCHAREST: NIRDMIC

Domestic and wild birds – main vertebrate hosts of West Nile virus

The seroprevalence of the antibodies against WNV was investigated on 1,480 domestic bird sera, and 563 wild bird sera from 17 species. The latter were collected in Bucharest metropolitan area (urban area and 2 rural locations in Giurgiu area) and in 4 localities of other 3 districts of Romanian Plain. The domestic bird sera (chicken, turkey, duck, and goose) were collected in 28 localities of the Bucharest metropolitan area (including the urban area), 36 localities of other 5 districts of Romanian Plain, 23 localities of Tulcea district in Dobrogea province and in one locality of Bacău district in Moldova province.

The general mean seroprevalence was 16.1% in domestic birds, and 9.9 % in wild birds.

It seems that the populations of mosquitoes were higher in rural habitats in comparison with natural ones this year when the climatic conditions continued to be unfavorable for mosquitoes; at least *Cx pipiens* larvae develop in artificial breeding sites existing in every rural courtyard near the domestic birds. On the other hand, the seroprevalence in domestic birds was higher in Tulcea district in comparison with the rest of the investigated areas.

Seroprevalence of WNV antibodies in wild and domestic birds (Romania. 2008)

	Wild birds		Domestic birds		Total	
Investigated areas in 2008	No. investigated / no. positive	Prevalence (%)	No. investigated / no. positive	Prevalence (%)	No. investigated / no. positive	Prevalence (%)
Romanian Plain	563 / 56	9.9	1123 / 159	14.8	1686 / 215	12.7
Bucharest Metropolitan Area	344 / 30	8.7	721 / 104	14.4	1065 / 134	12.6
Bucharest – urban	70 / 7	10.0	38 / 9	23.7	108 / 16	14.8
Ilfov – rural	-	-	613 / 80	13.1	613 / 80	13.1
Giurgiu - rural	274 / 23	8.4	70 / 15	21.4	344 / 38	11.0
Călăraşi district	-	-	51 / 0	0	51 / 0	0
Buzău district	-	-	100 / 12	12.0	100 / 12	12.0
Prahova district	125 / 18	14.4	27 / 4	14.8	152 / 22	14.5
Teleorman district	41 / 4	9.8	-	-	41 / 4	9.8
Dolj district	53 / 4	7.5	164 / 29	17.7	217 / 33	15.2
Olt district	-	-	60 / 10	16.7	60 / 10	16.7
Dobrogea	-	-	261 / 64	24.5	261 / 64	24.5
Tulcea district	-	-	261 / 64	24.5	261 / 64	24.5
Moldova	-	-	96 / 15	15.6	96 / 15	15.6
Bacău district	-	-	96 / 15	15.6	96 / 15	15.6
TOTAL	563 / 56	9.9	1480 / 238	16.1	2043 / 294	14.4

The species of wild birds investigated were: *Passer domesticus* (seroprevalence 15.2%), *Passer montanus* (26.7%), *Streptopelia decaocto* (12.9 %), *Columba livia domestica* (7.7 %), *Corvus frugilegus* (0%), *Perdix perdix* (15.2 %), *Phaseanus colchicus* (0%), *Dendrocopos major* (0%), *Hirundo rustica* (0%), *Sturnus vulgaris* (0%), *Lanius collurio* (0%), *Phoenicurus phoenicurus* (50.0%), *Pelecanus sp.* (25.0%), *Cygnus sp.* (0%), *Gallus sp.* (6.5%), *Anas sp.* (11.8%), *Anser sp.* (0%) (the last 5 exotic species in natural habitats in Bucharest Zoo).
The higher values of seroprevalence in Bucharest metropolitan area are as usually in sparrows (*Passer domesticus,* and *Passer montanus*). The general mean value of the seroprevalence of WNV antibodies in wild birds decreased constantly from 2005 (27.2 %) until this year (9.9) as a result of the general diminishing of the mosquito populations on very large areas in the country.
Sentinel birds (chickens – 134 and pigeons – 103) were installed in April in Bucharest Metropolitan area (urban and rural locations) and Tulcea. The rate of seroprevalence of antibodies against WNV increased until July – August and after this period the rate of seroprevalence slowly decreased. The preliminary investigation of chickens inoculated with West Nile virus showed that they produce antibodies and remain carriers of the virus which is present in saliva. The investigation will continue.

Horses – important tangential hosts of West Nile virus in Romania
The seroprevalence of antibodies against WNV has been investigated on 954 horse sera collected in 7 localities in Bucharest metropolitan area (including the urban area) and 8 localities of Tulcea district and its mean value was 25.2%. Here are the results.

Investigated area	Districts	# positive / investigated	Seroprevalence (%)
Bucharest metropolitan Area, in Romanian Plain	Bucharest – urban	1 / 36	2.8
	Ilfov - rural	46 / 294	15.6
Dobrogea	Tulcea	193 / 624	30.9
TOTAL 2008		240 / 954	25.2

The higher seroprevalence rate has been detected in Tulcea district. Previous studies had shown similar results. This shows the intense circulation of WNV in vertebrate hosts, in very large areas of Romania.

France: CIRAD
We received in spring from IZS (Italy) the last results of laboratory analyses of our serum samples collected from 2005 to 2007. Only 7 House Sparrows, two Black-billed Magpies and two Scops Owls out of ca. 3,300 birds (4,000 sera) tested positive for WNV antibodies. None of the migratory birds tested positive, including >900 Barn Swallows (including birds breeding in the Camargue region) and >200 *Acrocephalus* warblers. These data do not support the assumption that WNV is introduced to the Camargue by spring southern migrants or summer eastern migrants.
The very low seroprevalence we found in House Sparrows, together with the absence of seropositive birds in an additional outbreak site located out of the Camargue, is consistent with other studies. It suggest that House Sparrow plays a minor role in the WNV transmission cycle in southern France, related to either a low exposition to WNV vectors, or a low susceptibility to the circulating strain. Therefore, it is not a good indicator of WNV circulation.
Seroprevalence measured in Black-billed Magpie was also low, including in birds that had been potentially exposed to WNV during the outbreak in 2004. During our study, the only two seropositive magpies were both captured 5-8 km from the site of a confirmed equine case in 2004. These findings suggest that infection of Magpie by WNV is associated with epizootic events and that WNV did not circulate, at least in an extensive manner, beyond the area and period revealed by infected horses. However, our study reveals the existence of a transmission of WNV in the Camargue during a three-year period without any detectable emergence of WN fever in horses or humans.
Therefore, it seems that unfavourable conditions including virus strain, vector and reservoir competences or climate prevent the WNV to circulate at a high level for more than one summer and lead to only sporadic and geographically limited outbreaks in Southern France. A manuscript was written and submitted to the journal "Vector-borne and zoonotic diseases". The abstract, as submitted:
We investigated the seasonal and spatial patterns of WN virus activity over a three-year period in an area with a history of recurrent West Nile virus epizootics in Southern France. We conducted an intensive serologic

survey on several wild bird populations (>4000 serum samples were collected from 3300 birds) selected as potential indicators of the WNV circulation. West Nile virus antibodies were detected (by seroneutralization and/or plaque reduction neutralization) in House Sparrows, Black-billed Magpies and Scops Owls. Overall seroprevalence was low (<1%). including in birds that had been potentially exposed to the virus during recent outbreaks and House Sparrow appeared to be insufficient indicator of WN virus circulation. However, the detection of a seroconversion in one bird, as well as the detection of seropositve birds in all years of our monitoring including juveniles, indicate a constant annual circulation of WN virus at low level in this area, including in years without any detectable emergence of WN fever in horses or humans.

CZECH REPUBLIC: IVB

We collected and stored sera from 110 Czech horses and blood samples from 125 coots (*Fulica atra*); they will be examined by PRN microtest for the presence of WNV antibodies between December 2008 and and February 2009.

SPAIN: EBD

A new ELISA kit for WNV had been successful tested and validated again seroneutralization. The kit ID Screen, West Nile Indirect of ID-Vet, detects antibodies directed against the WNV. We observed a very high level of confidence. Unfortunately, the low serum volume availability from small passerine birds do not allow its use as a standard method for bird under 20-35 g or low volume samples .
Another new kit it is under testing. It need much less serum and seem to be very simple an efficient. It is expected to test it in the next 4 month. Near 1,000 birds sera from 2007 and over 2000 from 2008 are still pending to be analysed. Two month ago the National Analyses Lab and EBD Lab designed a well fit process to finish the whole stock before June 2008. During last 2 month had been analysed 1,158 sera. Highest prevalence was found in winter and spring (19% in January). The global prevalence for whole year round was 5.61%. [14.0% (2005); 14.7% (2006) and 5.6% (2007)]. *Turdus merula* (33.3%), *Fulica atra* (28.3%), *Passer hispaniolensis* (23.5%), *F. cristata* (19.2%), *Sylvia atricapilla* (18.2%), *Anas acuta* (17.7%) had the highest prevalence rates for a sample size over 11 animals. It is relevant to show than both migrant and resident species had high prevalence values.

Species	NEG	POS	TOTAL	PREV
Fulica atra	198	78	276	28.3
Plegadis falcinellus	144	10	154	6.5
Anas platyrhynchos	122	6	128	4.7
Gallinula chloropus	106	6	112	5.4
Fulica cristata	59	14	73	19.2
Corvus monedula	61	1	62	1.6
Passer hispaniolensis	39	12	51	23.5
Sylvia atricapilla	27	6	33	18.2
Ciconia ciconia	24	3	27	11.1
Platalea leucorodia	22	1	23	4.4
Anas acuta	14	3	17	17.7
Turdus merula	10	5	15	33.3
Sylvia borin	12	2	14	14.3
Luscinia megarhynchos	10	1	11	9.1

4.1. D WNV – 11. Reports on seroprevalence of WN antibodies by species, sex, age, habitat, and season

4.1.1. The total number of samples taken from wild birds in 2007

In the last report (November 2007), we had 810 samples taken from 19 wild birds species. Until the end of the year of 2007, we collected other 164 samples. The overall total is 974 samples taken from 22 wild-bird species in 2007 (Tables 1 and 2).

Tab. 1. Total number of samples taken from wild birds in 2007

Crt. no.	Bird species	No. of samples	Age and sex
1	*Phalacrocorax carbo*	12	Juveniles
2	*Larus cachinnans*	2	Juveniles
3	*Streptopelia decaocto*	8	Adults
4	*Cuculus canorus*	2	Adults
5	*Upupa epops*	4	Adults
6	*Picus canus*	1	Adult male
7	*Dendrocopos major*	1	Adult male
8	*Dendrocopos syriacus*	1	Adult male
9	*Lanius collurio*	15	Adults and juveniles
10	*Acrocephalus arundinaceus*	19	Adults
11	*Acrocephalus scirpaceus*	22	Adults
12	*Turdus philomelos*	4	Adults
13	*Turdus iliacus*	1	Adults
14	*Pica pica*	2	Adults
15	*Corvus monedula*	11	Adults
16	*Corvus frugilegus*	88	Adults
17	*Corvus corone cornix*	19	Adults
18	*Sturnus vulgaris*	56	Adults and juveniles
19	*Passer domesticus*	633	Adults
20	*Passer montanus*	61	Adults and juveniles
21	*Fringilla coelebs*	1	Female
22	*Emberiza schoeniclus*	11	Adults
	TOTAL	974	

The 974 samples were taken from birds caught in three study areas: Grindul Lupilor. Sălcioara and Mila 26 and in some other complementary areas: Iazurile, Sf. Gheorghe and Murighiol, where we found large aggregation of birds, being possible so to find here WNV.

Tab. 2. Sampling areas (for wild bird species) in 2007

Sampling areas	No. of samples
Grindul Lupilor	177
Salcioara	187
Maliuc-Mila 26	116
Iazurile	427
Sf. Gheorghe	14
Murighiol	53
TOTAL	974

4.1.2. Seroprevalence of WN on wild birds in 2007

Out of 974 blood samples taken from wild bird in 2007. We delivered to Cantacuzino Institute – Bucharest 158 samples and to IZS (Italy) 776 samples. The 40 remaining samples - taken in December of 2007, will be delivered to Teramo in December of 2008. The reported seroprevalence by Cantacuzino Institute was 11.5% for birds captured in 2007. Instead, IZS reported a very low seroprevalence, below 2% (15 positive samples out of 776 tested samples). These differences between seroprevalences will be discussed on the next EDEN Annual Meeting. However, the total tested samples (by both Cantacuzino Institute and IZS) taken from wild bird in 2007 is 837, the total positive samples is 22, i.e., 2.6% seroprevalence (Tab. 3)

Tab. 3. The total seroprevalence of wild bird in 2007

Crt. no.	Bird species	No of tested samples	No. of positive samples	Seroprevalence (%)
1	*Phalacrocorax carbo*	12	3	25.0
2	*Larus cachinnans*	2	0	0.0
3	*Streptopelia decaocto*	6	0	0.0
4	*Cuculus canorus*	1	0	0.0
5	*Upupa epops*	3	0	0.0
6	*Dendrocopos major*	1	0	0.0
7	*Dendrocopos syriacus*	1	0	0.0
8	*Lanius collurio*	15	1	6.7
9	*Acrocephalus arundinaceus*	14	0	0.0
10	*Acrocephalus scirpaceus*	12	0	0.0
11	*Turdus philomelos*	4	0	0.0
12	*Turdus iliacus*	1	0	0.0
13	*Pica pica*	2	0	0.0
14	*Corvus monedula*	7	2	28.6
15	*Corvus frugilegus*	53	5	9.4
16	*Corvus corone cornix*	18	2	11.1
17	*Sturnus vulgaris*	18	0	0.0
18	*Passer domesticus*	604	9	1.5
19	*Passer montanus*	52	0	0.0
20	*Fringilla coelebs*	1	0	0.0
21	*Emberiza schoeniclus*	10	0	0.0
TOTAL		837	22	2.6

Below we present the bird species with highest seroprevalence in 2007:
- *Corvus monedula* (Jackdaw) 2 seropositive samples out 7 tested; seroprevalence 28.6 %.
- *Phalacrocorax carbo* (Great Cormorant) 3 / 12; 25 %.
- *Corvus c. cornix* (Hooded Crow) – 2 / 11; 11.1 %.
- *Corvus frugilegus* (Rook) – 5 / 53; 9.4 %.
- *Lanius collurio* (Red-backed Shrike) – 1 / 15; 6.6 %.

4.1.2.1. The reported seroprevalence of WN on wild birds in 2007 – Cantacuzino Institute

Out of 158 samples delivered to Cantacuzino Institute, we got results only from 61 samples (Tab. 4). Out of 61 birds, 7 were positive (3 Great Cormorant, 1 Hooded Crow and 3 House Sparrow) – results a total seroprevalence of 11.5% (Tab. 4).

Tab. 4. Cantacuzino Institute lab results of the samples taken from wild bird in 2007

Location	Data	Species	Samples	Positive / tested	Observation
Tataru-Sf. Gheorghe	3-6.04.2007	*Phalacrocorax carbo*	14	3 / 11	Positive samples: 2, 8, 9
		Corvus c. cornix		1 / 2	Positive samples: 12
		Pica pica		0 / 1	
Iazurile	30.04–1.05.2007	*Passer domesticus*	47	3 / 41	Positive samples: 9, 22, 23
		Passer montanus		0 / 3	
		Streptopelia decaocto		0 / 2	
		Sturnus vulgaris		0 / 1	

4.1.2.2. The reported seroprevalence of WN on wild birds in 2007 – IZS–Teramo

In contradiction to ELISA test made by Cantacuzino Institute (showing the highest recorded seroprevalence in Europe), the sero-neutralisation tests made by IZS have shown a much lower seroprevalence (<2%, tables 5 – 7), still a bit higher than the European average. Different types of ELISA test are usual more sensitive and less specific than the sero-neutralisation methods, thus possibly explaining the observed discrepancies between the lab results. This will be discussed nuring the next EDEN annual meeting.

Tab. 5. IZS – Teramo lab results of the samples taken from wild bird in 2007

Results	*Plaque reduction neutralisation test*	*Virus neutralisation test*	Combined
Insufficient	363	2	1
Negative	403	594	631
Positive	10	9	15
Cytotoxic	0	77	37
Degraded	0	94	92
Total	776	776	776
SEROPREVALENCE	1.29%	1.16%	1.93%

Tab. 6. Wild bird species caught and found positive to WNV (sero-neutralisation methods), their sex, age, date, and place of catching

Crt. no.	**Bird species**	**Age / Sex**	**Data of catching day/month/year**	**Place of catching**
1	*Lanius collurio*	juv.	24/8/07	Iazurile
2	*Passer domesticus*	juv.	06/9/07	Sălcioara
3	*Passer domesticus*	□	25/9/07	Sălcioara
4	*Passer domesticus*	□	07/11/07	Maliuc - Mila 26
5	*Passer domesticus*	□	07/11/07	Maliuc - Mila 26
6	*Passer domesticus*	□	07/11/07	Maliuc - Mila 26
7	*Passer domesticus*	□	07/11/07	Maliuc - Mila 26
8	*Corvus frugilegus*	adult	21/11/07	Sălcioara
9	*Corvus frugilegus*	adult	21/11/07	Sălcioara
10	*Corvus frugilegus*	adult	22/11/07	Sălcioara
11	*Corvus c. cornix*	adult	26/11/07	Sălcioara
12	*Corvus frugilegus*	adult	26/11/07	Sălcioara
13	*Corvus frugilegus*	juv.	27/11/07	Sălcioara
14	*Corvus monedula*	□	10/12/07	Murighiol
15	*Corvus monedula*	□	10/12/07	Murighiol

Tab. 7. Wild bird species caught and found positive to WNV (sero-neutralisation methods)

Crt. no.	Bird species	Phenology	Age and sex	positive / tested birds	Prevalence (%)
1	*Lanius collurio* Red-backed Shrike	Migratory	1 juv.	1 / 15	6. 7
2	*Passer domesticus* House Sparrow	Resident	1 juv.. 2□. 3□	6 / 562	1. 1
3	*Corvus frugilegus* Rook	Resident. Winter visitor	1 juv.. 4 adults	5 / 52	9. 6
4	*Corvus c. cornix* Hooded Crow	Resident	1 adult	1 / 17	5. 9
5	*Corvus monedula* Jackdaw	Resident	1□. 1□	2 / 7	28. 6
	Total			15 / 776	1.9

As we see above in table 7, the highest seroprevalence (through sero-neutralisation methods) in 2007 was at Jackdaw (*Corvus monedula*) – 28.6% followed by Rook (*Corvus frugilegus*) – 9.6% Red-backed Shrike (*Lanius collurio*) 6.7% and Hooded Crow (*Corvus c. cornix*) – 5.9%.
A preliminary conclusion supervened after the examining of the table above is that all of the birds have been locally infected: 14 out of 15 being resident birds, and the only migratory bird (Red-backed Shrike) were juvenile at the moment of sampling. After the examination of all data gathered, we shall be able to find the proportion between migratory and the resident birds, also between juveniles and adults. The final results will be available when we receive all samples lab results.

Sampling from wild bird species in 2008

For 2008, we took 654 samples from 17 bird species (Tables 8 and 9). The number of samples decreased because it has been established that all EDEN-WNV teams must capture birds only in a single relevee (Figure no. 1 and 2). All 654 samples from 2008 (and 40 from 2007) are stored in DDNI-EDEN lab and are ready to be sent in December 2008 to IZS.
The sampling activity started on the 4th of January 2008 and ended on the 30th of October 2008.

Tab. 8. The total number of samples taken from wild birds in 2008

Crt. no.	Bird species	No of samples	Age and sex
1	*Ixobrichus minutus*	2	Adults
2	*Actitis hypoleucos*	1	Adult
3	*Upupa epops*	3	Adults
4	*Turdus merula*	4	Adults
5	*Turdus philomelos*	10	Adults
6	*Oriolus oriolus*	2	Females
7	*Lanius collurio*	3	Adult and juvenile
8	*Acrocephalus scirpaceus*	322	Adults and juveniles
9	*Acrocephalus arundinaceus*	91	Adults
10	*Corvus monedula*	6	Adults
11	*Corvus frugilegus*	56	Adults
12	*Corvus corone cornix*	3	Adults
13	*Sturnus vulgaris*	16	Adult
14	*Passer domesticus*	64	Adults and juveniles
15	*Passer montanus*	7	Adult
16	*Fringilla coelebs*	6	Adults
17	*Emberiza schoeniclus*	58	Adults and juveniles
TOTAL		654	

Tab. 9. Sampling areas (for wild bird species) in 2008

Samplin areas	No. of samples
Grindul Lupilor	589 (459 in the selected relevee)
Salcioara	0
Maliuc-Mila 26	0
Murighiol	65
TOTAL	654

We have sampled only two areas: 65 samples taken in the beginning of the year at Murighiol (crows caught in a cage) and 589 samples taken from April to October at Grindul Lupilor. Below you find the number of samples taken from target group species:

- Warbler group (*Acrocephalus scirpaceus* & *A. arundinaceus*) 413 samples;
- Sparrows group (*Passer domesticus* & *Passer montanus*) 71 samples
- Corvides group (*Corvus c. cornix* – Figure 3. *C. monedula* & *C. frugilegus*) 65 samples;
- Cormorant group (*Phalacrocorax carbo*) 0 samples.

Besides the target group species, we took samples from some complementary bird species:

- Reed Bunting (*Emberiza schoeniclus*) 58 samples.
- Starling (*Sturnus vulgaris*) 16 samples.
- Song Trush (*Turdus philomelos*. Figure 4) 10 samples.

Fig. 1 and 2. The selected Relevee on Grindul Lupilor

Fig. 3 and 4. Sampling a Hooded Crow (Corvus c. cornix) at Murighiol and a Song Trush (Turdus philomelos) at Grindul Lupilor

If last year we had 74 recaptures (birds with ring what were samples). in 2008 we took sample only from 28 birds caught with rings. Out of 28 birds, 15 had been caught in the selected relevee.
Birds caught with ring and sampled in 2008 belong to the following species:

- House Sparrow (Passer domesticus – 10 birds).
- Reed Warbler (Acrocehalus scirpaceus – 3 birds).
- Great Reed Warbler (A. arundinaceus – 10 birds)
- Rook (Corvus frugilegus – 1 bird)
- Starling (Sturnus vulgaris – 2 birds)
- Reed Bunting (Emberiza schoeniclus – 2 b.)

These 28 birds with possible serial sampling will be the subject of seroconversion (besides to the over 100 birds caught and sampled in 2006 and 2007). When we receive all the results of samples took from wild bird in 2006, 2007, and 2008, we will able to calculate the rate of seroconversion.

Sampling and seroprevalence of WN on wild birds, 2005-2008

Between 2005 and 2008, we took 2,680 samples from 31 wild bird species. The largest number of samples comes from House Sparrow (1,277 samples), Reed Warbler (357), Great Cormorant (239), Great Reed Warbler (206) and Rook (157). Over 50 samples come from Tree Sparrow (98), Starling (83), Reed Bunting (69) and Hooded Crow (55) – Table 10. Out of 2,680 samples, 1,889 (Tab. 11) were tested by Cantacuzino Institute – Bucharest and IZS. The overall seroprevalence for 2005 - 2007 is 10.4% (*n* = 1,889 tested samples), with 40% in 2005 (*n* = 5); 16.5% in 2006 (*n* = 1,047) and 2.6% in 2007 (*n* = 837) - Table 11.

Tab. 10. The total number of samples taken from wild birds in the Danube Delta Biosphere Reserve and Dobrudja Tableland in 2005, 2006, 2007, and 2008

Crt. no.	*Bird species*	*No. of samples*
1	*Phalacrocorax carbo*	239
2	*Ixobrichus minutus*	2
3	*Anas platyrhynchos*	11
4	*Fulica atra*	20
5	*Larus cachinnans*	2
6	*Actitis hypoleucos*	1
7	*Streptopelia decaocto*	11
8	*Cuculus canorus*	2
9	*Upupa epops*	7
10	*Picus canus*	4
11	*Dendrocopos major*	1
12	*D. syriacus*	1
13	*Lanius collurio*	25
14	*Lanius minor*	1
15	*Oriolus oriolus*	2
16	*Acrocephalus arundinaceus*	206
17	*A. scirpaceus*	357
18	*Turdus merula*	5
19	*Turdus philomelos*	14
20	*Turdus iliacus*	1
21	*Pica pica*	2
22	*Corvus monedula*	17
23	*Corvus frugilegus*	157
24	*Corvus corone cornix*	55
25	*Sturnus vulgaris*	83
26	*Passer domesticus*	1,277
27	*Passer montanus*	98
28	*Fringilla coelebs*	7
29	*Carduelis carduelis*	1
30	*Coccothraustes coccothraustes*	2
31	*Emberiza schoeniclus*	69
	TOTAL	2,680

Tab. 11. WNV seroprevalence in wild birds in the Danube Delta Biosphere Reserve and Dobrudja: 2005-2008

Year	No. of samples	No. of tested samples	No. of positive samples	Seroprevalence (%)
2005	5	5	2	40.0
2006	1,047	1,047	173	16.5
2007	974	837	22	2.6
2008	654	0	0	0.0
TOTAL	2,680	1,889	197	10.4

When we receive all the results of samples took from wild bird in 2006, 2007, and 2008, we will be able to calculate the total seroprevalence and the rate of seroconversion.

4.2. D WNV – 12. Reports on seroprevalence and sero-conversion rates in horses.

Seroprevalence and sero-conversion on horses in 2007

ITALY (IZSAM and ISS)

In 2007 we delivered 89 samples taken from previously WNV seronegative horses to IZS. Though we found a huge difference of lab test results between Cantacuzino Institute and IZS in birds, the seroprevalences were similar for horses. The seroconversion rate reported by IZS for those 89 previously WNV seronegative horses is 39.3% (35 seropositive samples out of 89 tested samples - Tables 12 and 13). Thus, in the horses, the ELISA test results (made by Cantacuzino Institute) are relatively similar to the sero-neutralisation tests results (made by IZS), and that allows us to process and interpret the results in an unitary way.

Tab. 12. The seroconvesion rate for the previously WNV seronegative horses in DDBR and Dobrudja Tableland – the second sampling in 2007

Results	*Plaque reduction neutralisation test*	*Virus neutralisation test*	Combined
Insufficient	2	0	0
Negative	54	54	54
Positive	33	35	35
Cytotoxic	0	0	0
Total	89	89	89

No seroconvertion or clinical illness in humans was recorded during 2008 in Padule di Fucecchio area. However, a considerable WN outbreak occurred in the Lombardy, Emilia Romagna, and Veneto of the Po river delta. This is the first outbreak in Europe since the start of EDEN and the first in Italy for 10 years. Outbreak data are still being analysed, but the table and map below illustrate the extent of the outbreak.

Tab. 13.

Province	Outbreaks	Outbreaks with clinical symptoms	Horses			Positive to lab test	
			Clinical cases	Total cases	Fatal cases	SN	PCR
Ferrara	62	11	17	125	2	226	19
Ravenna	4	0	0	4	0	4	0
Bologna	8	4	6	13	1	63	3
Modena	15	1	1	23	0	23	0
Rovigo	77	1	1	161	0	168	1
Padova	21	1	1	37	0	35	0
Venezia	8	0	0	20	0	20	0
Mantova	36	1	7	85	2	91	1
TOTAL	231	19	33	468	5	630	24

The following is the abstract of an article published in EUROSURVEILLANCE Vol . 13, Issue 48, 27 November 2008. In Italy the first occurrence of WNV infection was reported in Tuscany region during the late summer of 1998. In August 2008, the WNV infection re-emerged in Italy, in areas surrounding the Po river delta, and involving three regions Lombardy, Emilia Romagna and Veneto. WNV was isolated from blood and organs samples of one horse, one donkey, one pigeon (*Columba livia*) and three magpies (*Pica pica*). The phylogenetic analysis of the isolates, conducted on 255 bp in the region coding for the E protein, indicates that these isolates belong to the lineage I among the European strains. According to the analysis, both the 1998 and 2008 Italian strains as well as isolates from Romania, Russia, Senegal, and Kenya fell in the same sub-cluster.

ROMANIA. BUCHAREST: NIRDMIC

WNV seronegative horses sampling in 2008

The success of the finding of the seroconversion rate in 2007 has determined us to pay more attention on this issue, even the process is laborious and with a high rate of error. It is very difficult to take blood from the same horses, on the first hand because many horses are changed, sold and so on and on the other hand because in the Veterinary Direction lab are gathered many thousands samples. In 2008 we obtained 61 serum samples taken from previously WNV seronegative horses (in 22 places in DDBR and Dobrudja, Fig. 5), also to be sent to IZS in December 2008. Whether the previously lot had not been verified by us, in 2008, the serum samples of the seronegative horses were selected by us together with the veterinarians.

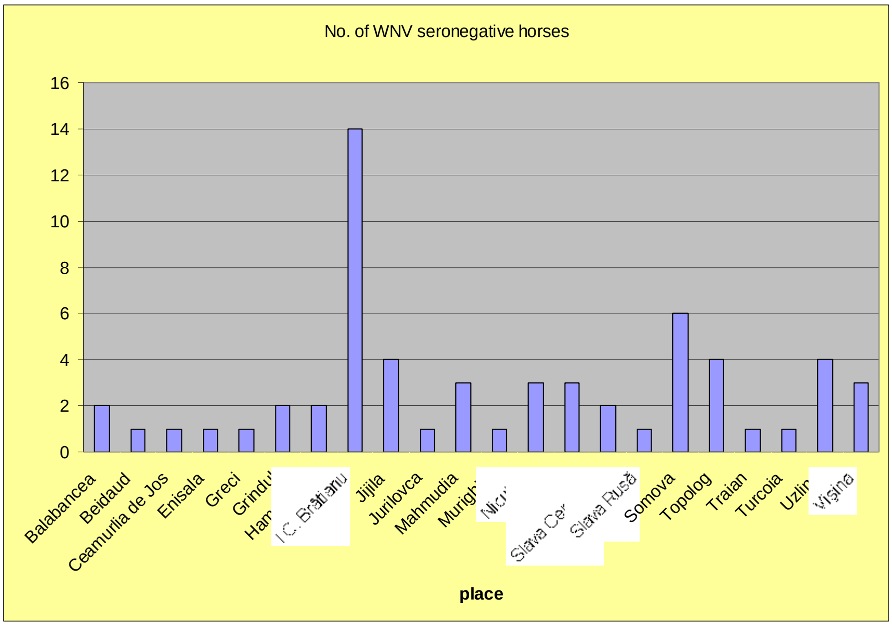


Fig. 5. Villages in DDBR and Dobrudjean Tableland where were collected in 2008 the serum samples from previously WNV seronegative horses

Ranges identifying and numerical evaluation of semi-wild horses in Danube Delta

For continuing of ecological and landscape approach and characterization of the diversity of reservoir and vector associations in space and time (horses database that will be integrated in GIS map. with all other layers) we have to deal with horse status in the study areas. We have researched the numbers, ranges, and habitats used by horses in Danube Delta Biosphere Reserve and Dobrudja Tableland. We have mentioned already that the number of effectives is changing every year, depending the most by meteorological conditions during winter, by number of foals, rate of survival of colts, food availability (in relation with hydrological conditions) and many other. Being at the beginning of this activity, we hope that we will have the support of some other EDEN teams. Experience with CIRAD' team cooperation (Ornithology and GIS teams) has been very important for us. In conclusion, for success of developing of this activity and its results integration we have to ask again for CIRAD team support.

Deliverables D WNV-11 Reports on seroprevalence of WN antibodies by species, sex, age, habitat and season (M42). A large amount of data has been collected by all teams. D WNV-12 Reports on seroprevalence and sero-conversion rates in horses. (M42). Not all study areas have horses for testing, but ample data has been collected in Romania and Italy. The outbreak in northern Italy will yield particularly interesting data. D WNV-13 Preliminary data and analysis of seasonal variations in composition and abundance of the avifauna in selected areas. (M42). A large amount of data is now available D WNV-14 Preliminary data and analysis of the role of birds in introduction and diffusion of the West Nile virus (M42). Question remains unanswered.
Milestones and expected result M WNV12 - Data over three seasons will indicate principal species that appear to be involved in transmission, and seroconversion rates of juveniles (M36). Good data is now available for all study areas. M WNV13 - Comparison of species data with mosquito capture data within and between study regions will build a picture of the bird/mosquito species involved in transmission, the habitats/mosquitoes associated with this transmission, and regional differences in transmission dynamics (M28). Good data is now available; awaits analysis. M WNV14 - Seroprevalence and seroconversion rates, combined with seasonal information from mosquito captures, will contribute to understanding of seasonality of transmission, which in turn may help interpret impact of variations between seasons, and indicate likely impacts of future climate change (M36). Data awaits analysis. M WNV16 - Seroprevalence in migratory birds will indicate species that are likely to be involved in intercontinental transport of virus, and may provide clues as to contact points between migratory and resident species (M30). Seroprevalence is low; conclusions may remain speculative M WNV20 - Data collected during the first seasons will contribute to epidemiological modelling and analysis (M36). Data awaits analysis.

WP WNV 5 – Data management and cross disciplinary modelling

Work package number	WNV5	Start date or starting event:			37			
Participant id	IPP	IVB	EID	CNM	IZS	CIRAD	NIRDMI	DDNI	Total
Person-months / participant	4	4	4	3	4	4	4	4	31

Objectives The general objective of this WP is to integrate information gathered through historical time series analysis and high resolution epidemiological studies in WP1-2-3-4 in order to construct area-wide statistical models for the occurrence of WNV in Europe. Now that a fair amount of data is available, more attention will be paid to this WP. During the second 18 months the specific objective will remain to be to integrate all collated data collected during the first two seasons. Preliminary epidemiological analysis outputs will allow the proposal of pathways for future modelling exercises and the improvement of field sapling protocols.

Work performed during previous reporting periods

Nearly all the teams have produced voluminous quantities of data, nearly all of which is in a form that must be processed before meaningful epidemiological analysis. As soon as Elisa- results are available, preliminary epidemiological analysis outputs will allow the proposal of pathways for future modelling exercises and the improvement of field sampling protocols. At this stage, maps of ecological units of relevance for mosquitoes and birds communities have been drawn up, and in coherence with the ability to discriminate these units from satellite images, and retrospectives populations of mosquitoes and birds have been evaluated. The diversity of the associations between reservoir and vector in space and time are explored. The approach his now well designed and has been developed in France, Italy and Romania.

- Substantial work has been accomplished to model vector abundance in Camargue, in collaboration with MAL and HIT HRRS. Several papers were published in good journals.
- A mathematical model of WN transmission was developed in collaboration with Utrecht University and IVB. The works was published in a good journal.

Description of work

The work has already been outlined in section 2 of this report. The emphasis will be on co-ordination of effort between participants, achieved by: (i) meeting at CIRAD headquarters in Montpellier, and (ii) work visits by members of the HIT team to each participant, to guide and facilitated processing of data. CIRAD, EID, NIRDMI, DDNI, IZSAM, IVB and EBD will be involved.

Achieved results:

ITALY (IZSAM and ISS)

See WP 1

ROMANIA. BUCHAREST: NIRDMIC

The analysis and evaluation of historical and present data for collaboration with Horizontal Team and for publication is in progress. As in the previous phases, DDNI team has (as the main activity for this WP) kept up-dated several databases and lists and contributed to the activities to be carried on by the Cantacuzino Institute (resulting so their databases and lists). One of the most important aspects is the contribution to the mapping of the mosquito habitats in the Danube Delta Biosphere Reserve and Dobrudjan Tableland (a collaboration of the Cantacuzino Institute, Danube Delta Institute, CIRAD, and EID).

Most of the information acquired from WP1-4 will be entered into geo-referenced multi-national databases (CNM, EID, CIRAD, IP, IZS, IVB, NIRDMI, and DDNI), developed with the help of the horizontal data management team, for environmental and spatial analyses. The remaining data will be sent by Coordinator to Horizontal Teams (on December 2008 and January 2009).

The analysis will seek to identify and quantify any significant associations (spatial and temporal) between occurrence of WNV (based on historical and current epidemiological data) and climate, ecology and other parameters. Because most of the data produced is already presented in this report (or were presented on the previous reports) we present below just a part of them:

- list of the investigated habitats for establish of the diversity of vector (mosquitoes) associations in space (Danube Delta Biosphere Reserve and Dobrudja Tableland) and time. It is important the mention that already all habitats were investigated.
- database of the diversity of reservoir – WNV significant ornithofauna in DDBR and DT – associations in space and time.
- list of the birds which we took samples in 2008, also, of the all birds caught and sampled in 2005, 2006, 2007, and 2008.
- lists with bird ectoparasites harvested in 2005, 2006, 2007, and 2008.
- lists with the mosquitoes collected in 2005. 2006. 2007 and 2008.
- seasonal dominance (2006. 2007 and 2008) of the mosquito fauna in the three study areas: Grindul Lupilor, Sălcioara and Maliuc – Mila 26.
- lists with the results of the birds' ectoparasites lab test.
- lists with the results of the wild bird serum samples – birds caught in 2005, 2006, and 2007.
- lists with the results of the horse serum samples – samples taken in 2005, 2006, and 2007.
- timeline graphs of hydrological variables (will be sent in January to the Coordinator after an entire year of recording).
- other data within WP 1, 2 and 4.

CIRAD-EID-IRD (WNV): the risk areas identified according to three scenarios of introduction will be compared with areas of virus circulation (equine cases. seropositive birds) during the last years. Land-use and climatic changes analysis will be performed to study the potential emergence years.

Using a landuse map linked to bird and mosquito abundance and diversity databases, the areas the most probable for WNV circulation was identified according to several scenarios resulting from different combinations of hypotheses concerning:

- introduction of the virus in the area: by migratory birds from Africa in spring; by migratory birds from East or Northern Europe in Summer; by overwintering mosquitoes;
- amplification of the virus: by mosquitoes (1 or 2 species) / by birds (magpies and sparrows; species listed by Jourdain et al. (2007); zones of high/low diversity; all species);
- spatial diffusion of the virus: magpies and sparrows; species listed by Jourdain et al. (2007); zones of high/low diversity; all species;
- emergence: by *Culex modestus* and *Culex pipiens*

From these numerous combinations, maps of potential circulation zones were mapped, highlighting strong spatial heterogeneities; they will be compared with known areas of virus circulation in the equine populations.

CIRAD-DDNI (WNV): distribution maps of birds species potentially involved in the WNV transmission processes will be produced. Impact of bird diversity and abundance on the WNV circulation will be studied and results will be compared with the french area.

In collaboration with the Danube Delta Institute (DDNI), maps of the distribution of bird species were created, based on a land cover map derived from Landsat ETM+ imagery and a Bird Database developed by the DDI. Richness and Diversity indices were also calculated. The validation of these maps is in process. This step is necessary to go further into the analysis.

Deliverables D WNV-15 Preliminary epidemiological analysis of the first two complete transmission seasons (2006 and 2007) (M42). A major effort on analysis is now due
Milestones and expected result M WNV17 - Data collected during in the first 24 months will contribute to epidemiological modelling and analysis. This will be critical for planning of the next 18 month period (M42). Data analysis now due, including studies encompassing all teams.

Workpackages MAL1 to MAL5: Malaria

Deliverable review

					EID 10	ISS 12	IRD 13	HUESRL 14	IHMT 15	INH 16	UVEG 17	NHM 34	NIRDMI 43	IPA 46
Work pak	Del. no.	Deliverable name	programmed delivery month	effective delivery month	France	ITALY	France	TURKEY	Portugal	MOROCCO	SPAIN	UK	ROMANIA	ALGERIA
WP1	D MAL 1	Report of environmental parameters	48		D	C	D	D	D	C	C	NR	D	D
WP1	D MAL 2	Spatial database on environment in every selected area.	48		D	C	D	D	D	C	C	NR	C	C
WP1	D MAL 3	Standardized description of environment and climate into the model areas	48		D	C	D	D	D	C	C	NR	C	C
WP1	D MAL 4	environmental map of model areas	48		E	B	E	D	D	C	C	NR	B	C
WP2	D MAL 5	Report of past and current anopheline species in selected model areas	48		D	D	D	D	D	C	D	NR	D	D
WP2	D MAL 6	Database on anopheline collections in model areas.	48		NR	D	E	D	D	D	D	NR	D	D
WP2	D MAL 7	Report and articles on biology and vectorial role of anopheline populations	48		D	D	D	D	D	D	D	NR	D	D
WP2	D MAL 8	report on anopheline systematics and population genetics.	48		D	C	D	D	D	C	C	C	D	C
WP2	D MAL 9	report on vector competence.	48		NR	B	D	D	D	B	B	NR	A	C
WP3	D MAL 10	Report on current and past autochthonous and imported malaria	48		NR	D	D	D	C	D	C	NR	D	D
WP3	D MAL 11	Report on human movements in relation to malaria importation/exportation	48		NR	C	D	D	C	C	C	NR	C	D
WP3	D MAL 12	Report on the relationship between modifications of environment/human activities and malaria/potential vectors	48		NR	B	D	D	C	C	B	NR	C	D
WP5	D MAL 13	draft of geo-referenced database on environment, vectors, malaria, and public health.	48		D	D	E	D	C	C	B	NR	A	D
WP5	D MAL 14	draft of maps and risk models in selected areas.	48		D	C	D	C	C	B	B	NR	A	C
WP5	D MAL 15	Bibliographic database.	48		NR	C	D	C	C	C	C	C	C	C

Table MAL-01. Deliverable status per MAL partner

Executive summary

In 2008, all the teams focused on completing the field data bases, which now cover 4 project years, the development of models of mosquito distribution and risk based on these data sets, and on the publication of the obtained results. Mosquito parameters have been recorded in all regions from the project. Most of the partners report the development of a GIS including environmental data. However, such tools remain to be adopted by some partners.

Molecular and population genetics studies have been conducted on *Anopheles atroparvus*, *An. labranchiae* and *An. hyrcanus* through the EDEN MAL collaborative network, and results will be presented during the 2009 Annual meeting. Vector competence has been assessed by experimental transmission of an African *Plasmodium falciparum* strain, in Nijmegen. Almost all partners have sent mosquito populations to Nijmegen to be tested. All species but *An. hyrcanus* were able to replicate *P. falciparum*. However, rates of infection were generally low compared to highly susceptible control strain, and depend of species and rearing conditions. Nijmegen subcontract partner has already tested all the mosquitoes as initially planed. Some additional mosquito populations remain to be tested: i.e. Romania, Algeria, Morocco, and Spain. Since these cannot be tested in Nijmegen a satisfactory alternative solution was found at the Pasteur Institute in Paris. In 2008, *An. labranchiae* from Corsica en Italy was tested by IP, and it is agreed to test more in 2009.

The studies related to disease, human health, human migration and imported / autochthonous malaria made good progress during year 3 and 4 and are almost finished in some countries.

Epidemiological R_0 models remain under development (finished in France, initiated in Spain, Algeria, Turkey, Morocco), as well as multi agent models (finished in France, initiated in Portugal). Ideally, these approaches are developed in collaboration with the relevant HITs (horizontal teams). However HIT don't have the capacity to collaborate with all (the 40 or so) vertical teams, and data collected by these vertical teams are not always robust enough for developing models.

The malaria networks are fully operational. Several scientists and students have visited other partners. Many posters and communications related to EDEN MAL have been presented during national and international meetings. Many scientific papers related to EDEN MAL have been published or submitted during year 4. Two EDEN MAL PhD thesis have been finalized in 2008.

Publications

The following PhD students have completed their thesis at least partially using EDEN data:

Ponçon Nicolas, EDEN-MAL France, Etude des risques de ré-émergence du paludisme en Camargue. (Defended the 28th of January 2008)

Sousa, CA (2008). PhD Thesis: Malaria vectorial capacity and competence of Anopheles atroparvus Van Thiel, 1927 (Diptera: Culicidae): Implications for the potential re-emergence of malaria in Portugal. "Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa":187pp.

The following PhD students are currently conducting research using EDEN data:

César Capinha, "O mosquito vector da malária Anopheles atroparvus Van Thiel, 1927 (Diptera, Culicidae): adequabilidade de habitat para Portugal continental e potenciais alterações futuras do seu espaço climático" MSc. Geographic Information Systems and Modelling applied to Planning – Faculdade de Letras – Universidade de Lisboa.

Eduardo Gomes: "Potential risk of malaria transmission in Portugal mainland"; MSc. in Territorial Managing – specialization in Remote Sensing and Geographic Information Systems. – na Faculdade de Ciências Sociais e Humanas – Universidade Nova de Lisboa

José Manuel Latorre Estivalis, "Riesgo de re-introducción del Paludismo en la costa mediterránea española: El Delta del Ebro y La Albufera de Valencia" Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia (Valencia, España).

MARIA ANGELES ZURIAGA A fellowship from (1 April 2007 - 31 October 2008) to carried out studies on: anophelinae population biology and historical data on Malaria.

Publications of EDEN-MAL (with an EDEN number)

ALTEN B, KAMPEN H, FONTENILLE D, 2007. Malaria in Southern Europe: resurgence from the past?: Emerging Pests and Vector-Borne Diseases in Europe (W Takken and BGJ Knols, Eds.). Wageningen Academic Publishers, Wageningen, the Netherlands. p 35-58

FARAJ C., ADLAOUI E., BRENGUES C., FONTENILLE D., LYAGOUBI M. Résistance de An. labranchiae au DDT au Maroc: Mise au point, identification des mécanismes et choix d'un insecticide de remplacement. La Revue de Santé de la Méditérranée Orientale (in press) EDEN0024

FARAJ C, ADLAOUI E, OUAHABI S, RHAJAOUI M, FONTENILLE D, LYAGOUBI M, 2009. Entomological investigations in the region of the last malaria focus in Morocco. Acta Trop 109: 70-73 'EDEN0114

FARAJ C, OUAHABI S., ADLAOUI E, BOCCOLINI D., ROMI R., EL AOUAD R., 2008. Etude du risque de réémergence du paludisme au Maroc: analyse du potentiel paludogène d'une zone rizicole au nord du pays. *Parasite* 15, 4: 605-610. EDEN0103

DI LUCA M., BOCCOLINI D. , SEVERINI F., TOMA L., MANCINI BARBIERI F., MASSA A., ROMI R., 2008.A two-year entomological study of potential malaria vectors in Central Italy. Submitted to Vector-Borne and Zoonotic Diseases. EDEN 118

LOURENÇO P., LOPES P., SEIXAS J., SOUSA C., NOVO T., RODRIGUES J., ALMEIDA P. Malaria Vector Density Modelling using MODIS NDVI. Remote sensing of Environment (accepted) EDEN 0062

MELERO-ALCIBAR R., MOLINA R., MORCHON R., SIMON F., LATORRE-ESTIVALIS J.M., BARGUES M.D., MAS-COMA S. BAEZ M., 2006.- Actualización de la fauna de Culícidos de las Islas Canarias: Isla de Gran Canaria. Boletín de la SEA, submitted.

PONÇON N., TOTY C., L'AMBERT G., LE GOFF G., BRENGUES C., SCHAFFNER F., FONTENILLE D. Biology and dynamics of potential malaria vectors in Southern France. *Malaria Journal*, 6: 18. EDEN0038.

PONÇON N., TOTY C., L'AMBERT G., LE GOFF G., BRENGUES C., SCHAFFNER F., FONTENILLE D. Population dynamics of pest mosquitoes and malaria and West Nile virus potential vectors in relation to climatic factors and human activities in the Camargue – France. *Medical and Veterinary Entomology*, 2007, 21: 350-357. EDEN0061

PONÇON N., TOTY C., LANGEWIESCHE K., DERVIEUX A., FONTENILLE D., 2008. Étude du risque de ré-émergence du paludisme en Camargue: nécessité de l'interdisciplinarité. In: Bley D, Gruénais ME, Vernazza-Licht N (eds), Milieux de vie et santé. Quelles pratiques interdisciplinaires. EDISUD (coll. Ecologie Humaine). In press.

PONÇON N., TOTY C., KENGNE P., ALTEN B., FONTENILLE D., 2007. Molecular evidence for similarity between Anopheles hyrcanus (Diptera: Culicidae) and Anopheles pseudopictus (Diptera: Culicidae), sympatric potential vectors of malaria in France. *Journal of Medical entomology*, 2008, 45 (3), p. 576-580. EDEN0077

Ponçon N, Tran A, Toty C, Luty A, Fontenille D, 2008. A quantitative risk assessment approach for mosquito-borne diseases: malaria re-emergence in southern France, Malaria Journal, 7:147 EDEN0106

TRAN A., PONÇON N., TOTY C., LINARD C., GUIS H., FERRE JB., LO SEEN D., ROGER F., DE LA ROCQUE S., FONTENILLE D., BALDET T., 2007. Use of remote sensing to map larval and adult populations of Anopheles species in Southern France. *International Journal of Health Geographics* 2008, 7 (9), p. 26, EDEN0059

PONÇON N., BALENGHIEN T., TOTY C., FERRÉ JB., THOMAS C., DERVIEUX A., L'AMBERT A., SCHAFFNER F., BARDIN O., FONTENILLE D., 2007. Effects of local anthropogenic changes on potential malaria vector Anopheles hyrcanus and West Nile virus vector Culex modestus, Camargue, France, *Emerging Infectious Diseases*.13: 1810-1815. EDEN0056

SAINZ-ELIPE S., BARGUES M.D., LATORRE J.M., MASIA M., ESCOSA R., FUENTES M.V. & MAS-COMA S., 2006.- Análisis del riesgo actual de transmisión de malaria en zonas históricamente endémicas de España: el Delta del Ebro (Tarragona, España). Acta Tropica, submitted.

LINARD C., PONÇON N., FONTENILLE D., LAMBIN E., 2008. A multi-agent simulation to assess the risk of malaria re-emergence in southern France. Ecological Modelling, in press. EDEN0107

Publications of EDEN-MAL (EDEN number not applied for)

DOUDIER B., BOGREAU H., DEVRIES A., PONÇON N., STAUFFER W., FONTENILLE D., ROGIER C., PAROLA P., 2007. Can malaria be back in Southern France? Autochthonous malaria from Marseilles to Minneapolis *Emerging Infectious Diseases* 13: 1236-1238.

MORCHON R., BARGUES M.D., LATORRE J.M., MELERO-ALCIBAR R., POU C., MAS-COMA S. & SIMON F., 2007.- Culex pipiens is a natural vector of Dirofilaria immitis in an endemic area of Western Spain. *Vector-Borne Parasitic Zoonoses*, 7 (4): 653-658.

MAS-COMA S., VALERO M.A. & BARGUES M.D., 2007.- Effects of climate change on animal and zoonotic helminthiases. In: Impact of Climatic Changes on the Epidemiology and the Control of Animal Diseases (S. De La Rocque ed.). Scientific and Technical Review, World Organization for Animal Health (OIE), Paris. *Revue Scientifique et Technique de l'Office Internationale des Epizooties*, 7(2): 443-5.

MELERO-AlLCIBAR R., MOLINA R., MORCHON R., SIMON F., LATORRE-ESTIVALIS J.M., BARGUES M.D., MAS-COMA S., POU-BARRETO C. & VALADARES B., 2007.- Nota sobre la fauna de Culícidos de las Islas Canarias II: Isla de Tenerife. *Boletín de la Sociedad Entomológica Aragonesa*, 42: 463-464.

Meetings where papers involving EDEN-MAL ideas have been presented

LATORRE J.M., BARGUES M.D. & MAS-COMA S., 2008.- A compared entomological and historical study of two past malaria endemic areas in Spain. Xth European Multicolloquium of Parasitology (Paris, France, 24-28 August, 2008). Program & Abstract Book, P-0150: 126.

FONTENILLE D., 2008. Vecteurs, climat, environnement et mondialisation dans l'évolution du Chikungunya et du paludisme en Europe (conférence plénière invitée). Congrès international d'épidémiologie. Paris, 10-12 septembre 2008.

BARGUES M.D., LATORRE J.M., MORCHON R, MELERO R., POU-BARETO C., MOLINA R., SIMON F. & MAS-COMA S., 2008.- Characterization of Anopheles populations in Southern Europe by means of ribosomal RNA sequences. XVIIth International Congress for Tropical Medicine and Malaria-ICTM (International Convention Center, Jeju, Korea, 29 September-3 October, 2008). Program & Abstrac Book, P436: 58 y CD-ROM.

MORCHON R., BARGUES M.D., LATORRE J.M., POU-BARETO C., MELERO R., MOLINA R., MAS-COMA S & IMON F., 2008.- Culex pipiens (haplotype H1) implicated as potencial natural vector of Dirofilaria immitis in Salamanca (endemic area of western Spain). XVIIth International Congress for Tropical Medicine and Malaria-ICTM (International Convention Center, Jeju, Korea, 29 September-3 October, 2008). Program & Abstrac Book, P519: 61 y CD-ROM. ISSN: 0023-4001 ON LINE ISSN: 1738-0006.

OZER N., 8th Ecology and Environment Congress. 20-23 October, Cyprus. Remote Sensing and GIS based ecosystem analyses: Eden Project, Field Site Sanliurfa, Turkey.

WP MAL 1 – Landscape, biotopes and habitats

Work package number	MAL1	Start date or starting event:		37			
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	4	3	4	3	3	3	4
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	0	6	3				29

Objectives

The general objective of this WP is to characterise at a high resolution (30m), dominant ecosystems and environmental parameters linked to Anopheles vectors. During the next 18 months, the scientific objective include further improvements in the landscape description of the model regions, in secondary model areas and in other relevant Mediterranean ecosystems

Work performed during previous reporting periods

Vector and Human host habitats have been described in the studied model areas Algeria (Ouargla), France (Camargue), Italy (Maremma), Morocco (Province of Chefchaouen), Portugal (Troia Peninsula), Romania (south of Bucharest), Spain (Ebro Delta), Turkey (Sanl?urfa province) and climatic data have been extracted at a regional basis. Same work has been initiated in second model areas: Algeria (El Tarf), France (Corsica), Italy (Delta of the Po River), Morocco (Larache), Romania (Danube Delta), Spain (L'Albufera de Valencia and Murcia). The first GIS version are available for most of the countries and some analyses between climate data and mosquito densities (based on historical data set) have been proceeded. First generation of models is under construction, notably in France and Portugal.

Description of work

Algeria: To analyze the environmental parameters and compare with results obtained from field (entomological data) in the second model area (El Tarf) using satellite imagery.

Achieved results:

During the period from 1/11/07 to 31/10/08, the description of the landscape, biotope and habitat of the two model regions (El Tarf in north and Ouargla in south of country) were finalized. In addition an extended area, Tinzaouatine, a small village close the Algerian-Malian border (19°.95' N; 02°.95'), where an outbreak of malaria occurred in 2007, was described. For more spatial details a satellite image was provided by the Algerian Space Agency.

Fig. MAL01 – Panoramic view of the Tonga lake showing the eutrophication of the basin.

The effects of eutrophication growth on malaria vector abundance were assessed in the National Park of El Kala between April to November 2007, and in the summer 2008. Despite the eutrophication of the Lake, *Anopheles labranchiae* is still present in this area producing in the end of summer an important anophelism. The vectorial capacity exhibited a seasonality variation. However, the intensive eutrophication may have a negative impact on the population dynamics of *An labranchiae* in future.

France: Environmental data concerning the second model area (Corsica) will be completed.

Achieved results: Concerning the Corsica Island, where malaria occurred until 1972, a recent survey has been conducted on anopheline mosquitoes and malaria risk by teams from the University of Corte and French InVS. Bibliographical data were explored to plan a field study in this area.

Field data on environment (ecological level based on vegetation pooling, land cover, soil, vector habitats: breeding sites, biotopes of the adults) has been collected. GIS is under construction.

Spain: To continue the landscape, habitats and biotopes characterization with remote sensing data in the Albufera (Valencia) and Murcia region to elaborate the GIS. To process and elaborate the climodiagrams of the Murcia region to compare with the other studied areas. To start the biotope sampling and climatic analyses in Andalucía and Extremadura provinces, another past endemic area of Spain. To obtain historical data from climatic stations and biotope characterization including landuse, vegetation, topography, etc. of Andalucia and Extremadura provinces. To extend the Index of Potential Transmission of Malaria to other parts of Spain. To obtain more remote sensing data and historical climatic data from other past endemic areas.

Achieved results: During 2008, the study has been conducted in the Ebro Delta (Tarragona, Catalonia), in order to obtain the entomological data necessary for completion the population dynamic study developed during 2005-2006. The presence of *An. atroparvus*, the available resources facilities, the complete GIS developing and the great knowledge of Ebro Delta explain the return to this area.

Italy, Morocco, Portugal, Romania and Turkey will complete environmental data recoding and processing, particularly when a second model area has been selected. Except in Portugal satellite images will be acquired, processed and the landscape classification outputs will be validated.

Achieved results:

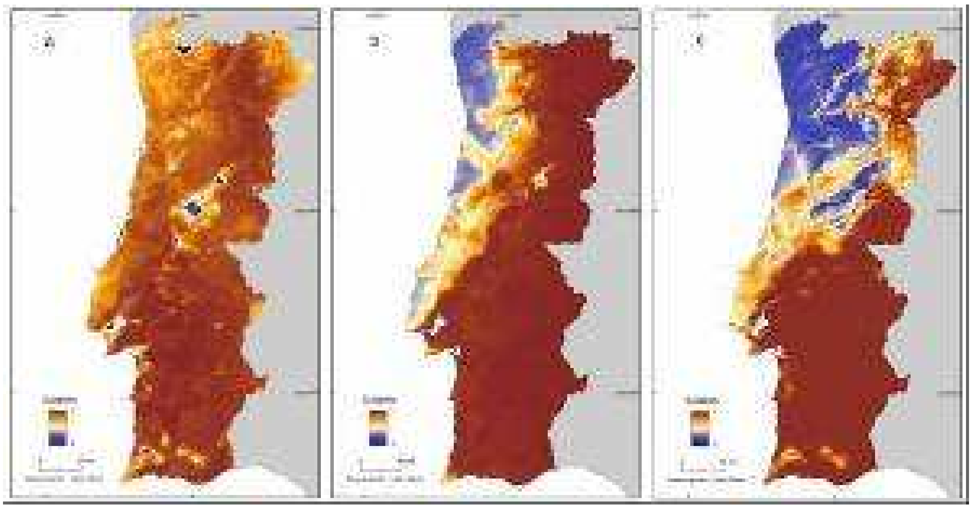


Figure MAL02 Habitat suitability for *Anopheles atroparvus* in Portugal obtained using different correlative methods:

a) Mahalanobis distance

b) Logistic regression

c) Artificial neural network

Italy: The environmental study of the coastal and inland parts of Maremma Region, Grosseto province started in 2005 and completed in 2007, was extended in 2008 to the southern part of the study area (Viterbo province).

Morocco: Institut National d'Hygiène de Rabat in collaboration with laboratoire de botanique de la Faculté des Sciences de Rabat have developed RS and GIS tools in the area of public health. Within the frame work of EDEN research work focused on Chaouen and Larrache areas.

Portugal: In order to model the *An. atroparvus* density in Portugal mainland, an occurrence suitability map was developed, taking into consideration a set of environmental factors. This was carried out by defining first the input variables of the model, testing several predictive models (Fig. MAL02), their accuracy, and then proceeding toward a final suitably model.

Romania: The important feature of the climate in Romania in the last century was the constant increase of the mean temperature over the entire country (mean value of the warming was 0.3 ºC); this process was most marked in the southern and eastern half of the country (0.8 ºC at Bucharest and the rest of the eastern part including also Moldova province).

These climatic conditions influenced the mosquito fauna including the anopheline populations in some areas and moments.

In Comana area including the Comana lake and in the Danube delta and lagoons both areas with permanent breeding sites for mosquito larvae the anopheline larvae develop even during periods when the climatic conditions are not that favourable.

Graphs showing daily climatic data (minimum, mean and maximum temperatures, quantity of precipitations, relative humidity, cloudiness and wind speed) in Romanian Plain (Comana area) and Dobrogea Province (the second study area) from the National Meteorological Administration for 2007 were obtained and analyzed.

The data including geology, hydrology, types of ecosystems, human habitat, climate of the second study area in Dobrogea province (the Black Sea coast with the highest frequency of severe malaria cases and the presence of two main vectors, *Anopheles sacharovi* and *Anopheles atroparvus* in the south and the area of "anophelism without malaria" in Danube Delta to the north) were collected for the description of this area.

Deliverables
D MAL01 – Report on environmental parameters linked to anopheles vectors and malaria parasites, particularly in the second model areas (M 48). Completed in first model areas in France, Algeria, Turkey, Portugal; Italy; almost finished in first model area Romania, Spain and Morocco; ongoing in Corsica (France), El Tarf (Algeria), Danube delta (Romania) and in Larache (Morocco), D MAL02 – Spatial database on environment in every selected area (M48). Completed in first model area in France, Algeria, Portugal; on going in Turkey; Italy, Romania, Spain and Morocco; D MAL03 – Standardized description of environment and climate into the model areas (M48). Standardization remains to be done properly. Some partners have not standard data (some data missing, some other not necessary) D MAL04 – Draft of environmental map of model areas related to anopheles breeding sites in model areas (M48) Completed in first model area in France, Algeria, Portugal, on going in Romania, Spain, Turkey; Italy and Morocco
Milestones and expected result
M MAL04 – Collected raw data from first model areas is available and enable to create spatial data layers to be included in the GIS (M 48). M MAL05 – Preliminary GIS model enable first spatial analysis of vector / Malaria/environment associations finished only in some partners (France), other ongoing (M 48). M MAL17 – Collected raw data related to second model area are available (M 48). M MAL18 – Detailed environmental information about second model areas is available (M 48).

WP MAL 2 – Vector bionomics and competence

Work package number	MAL2	Start date or starting event:				37	
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	15	10	15	12	10	8	17
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	5	30	16				138

Objectives

The general objective of this WP is to study the population ecology of anopheline larvae and adult populations in Europe in order to estimate the mosquitoes' vectorial capacity and competence. During the second 18 month period of the project the specific objectives are:

- To complete the recording of historical data on the distribution and bionomics of anopheline species;
- To conduct population dynamics studies;
- To continue the vector competence studies on potential vector;
- To characterise species and populations in the *Anopheles maculipennis*, *An. claviger*, *An. sergentii* and *An. hyrcanus* complexes and their distribution.

Work performed during previous reporting periods

A review of the literature and historical reports from Ministries of Health, research institutes and mosquito-nuisance control agencies was conducted to obtain historical data on the distribution and bionomics of Anopheline species, in each partner country. Mosquitoes have been collected by various methods depending on location, mosquito biology, and feasibility; a huge amount of data on all these parameters is available. Multiplex PCR for identifying species from Maculipennis complex has been improved and evaluated in different countries. Several rDNA sequences from specimens from Maculipennis complex and An. hyrcanus from different countries have been obtained. Vector competence of Anopheline populations from Portugal, Italy, France, Algeria, Morocco and Turkey has been assessed. The transmission of the African NF54 *Plasmodium falciparum* strain has been experimentally proved with different colonies, including *An. atroparvus* from Portuguese and France, *An. pseudopictus* from Turkey, *An. melanoon* and *An. hyrcanus*. Only *An. hyrcanus* from France was unsuccessfully infected. Studies on Anopheline populations (genetic structures, gene flow, etc ...) have been initiated by collecting specimens from *An. atroparvus* and *An. labranchiae*, and selecting markers (mt and ITS2 DNA and microsatellites loci.

Description of work

In all countries where a second model area has been selected, mosquito population studies will continue. In each country entomological data will be obtained at a country wide scale using: literature, in-country collaborations, and localised field sampling. Potential (or effective) vectors identified during years 1-3 will be studied in detail. Vector competence of species and populations (*An. melanoon*, *An. atropravus*, *An. Labranchiae*, *An. Multicolour*, and *An. hyrcanus*) will continue through collaboration with Nijmegen. Population genetic study and morphometric study of *An. labranchiae*, *An. hyrcanus* and An. atropavus will be continued involving collaboration between all partners depending of the presence of species in their country. In all countries the bibliographic data base will be finished and shared between all partners via the EDEN Web site.

Algeria: ELISA-CSP and ELISA- blood meal tests will be carried out, and the vector competence of *An. multicolor* will be assessed.

Achieved results: ELISA-CSP and ELISA- blood meal tests have been carried out. Sampling of mosquitoes was extended to other areas and new species were caught for the first time in Algeria: *Anopheles gambiae sl* (Gilles) and *Culex quinquefasciatus* in the south of the country, in Tinzaouatine, and *Coquillettidia sp*. in the North of the country (Tonga Lake, El Kala, 8°30' E, 36°51' N). The first report of *An. gambiae* in Algeria was presented at X^th^ Multicolloqium of Parasitology (Paris, August 2008). The theoretical vectorial capacity of *An. labranchiae* and *An multicolor* was evaluated in El Kala and in Ouargla respectively. The highest values were in June for *An. multicolor* (VC = 10,7 for *P. vivax* and 9,1 for *P. falciparum*), and in September for *An. labranchiae* (Vc = 3.6 for *P. vivax* and 2,4 for *P. falciparum*). Feeding preference of *An. labranchiae* was studied on females collected indoor and in animal shelters, around the Tonga lake. The test was performed by ELISA. The human blood index and the human biting habit were 0.28 and 0.09 respectively.

The preliminary results of the studies of the biology of malaria vectors (*An. labranchiae*) in the Tonga Lake, and the biology of *An. multicolor* in Ouargla were presented in the X^th^ Multicolloqium of Parasitology (Paris, August 2008).

Study of the population genetics of *An. labranchiae*: specimens were sent to the LIN (Montpellier) and to the NHM (London) for DNA sequencing and data analysis (alignment of sequences and construction of the phylogenetic tree) are in progress. We are awaiting the final results.

Vector competence of *An. multicolor*: not yet achieved.

France: entomological investigations focused on *An. plumbeus* will be continued. Entomological investigations in Corsica will be developed. New collection of mosquitoes will be organized.

Achieved results: bibliographical and historical data on malaria vectors in Corsica were analyzed in order to i) increase our knowledge on anopheles distributions, ii) define precisely some study areas, iii) prepare field activities. During the first field session, Corsican partners were met and the basis for collaboration was set. This enabled to select interesting breeding sites and potential resting places of anopheles vectors.

Field entomological sessions (including captures by "Mosquito Magnet", resting fauna captures, human bait catch and larvae prospection) were carried out in Corsica in 4 different areas for identifying Anopheles species and their aggressiveness against human. *An. claviger sl*, *An. Melanoon,* and *An. labranchiae* were found in different biotopes (marshes, reed beds and riversides), the last two species are antropophilic. A total of 400 *An. labranchiae* were sent at Pasteur Institute in Paris to conduct experimental transmission trials with culture *P. falciparum* strains (NF54). About 30% of the female were able to develop one (or maximum two) oocystes, suggesting that they should be able to transmit but are not good vectors.

The genetic structure of *An. labranchiae* populations from Italy, Morocco, Algeria and Corsica was analysed. Result should be published in 2009.

Morphometric studies initiated in year 2 on *An. hyrcanus* were extended through collaborations with EDEN MAL partner (Turkey). Last samples are currently being processed for molecular biology analysis.

Spain: Research of location and analysis of new biotopes where Anopheles are present in the Murcia region. Study of the biology of these populations.

Achieved results: historical data (entomological studies, Anopheles species presence and location) have been analyzed in three regions. The available entomological data for these regions started from the middle of the previous century giving great relevance to the study.

Mosquito Biology: The plan to develop investigations in Murcia region has been cancelled in order to better focus on Ebro delta where yhe entomological study has been completed (Tarragona). The objectives were to determine the parity rate, resting places, aggressiveness and feeding and overwinter behaviour of *An. atroparvus* and human baiting rate in order to complete the entomological study developed in 2005-2006 in the Ebro Delta. A total of nine field expeditions were conducted from June to October. The feeding behaviour is being analyzed in 193 *An. atroparvus* females collected in biotopes with different animals, using the ELISA technique. The technique has been implemented and developed in the Department of Parasitology for the first time after training in LIN during October 2005. According to the preliminary results, blood from horse, pig and hen are abundant and the human blood could be a percentage between 5-10% of the samples.

The vector capacity is being estimated in 3 areas of the Ebro Delta. The longevity of *An. atroparvus* females, the length of the gonotrophic cycle for *Plasmodium vivax* and *P. falciparum*, and the Pn for both Plasmodium species have been obtained.

The factors that determine the dynamics of *An. atroparvus* in the Ebro Delta have been studied. The rice cultivation cycle, the temperature and precipitations of April and May and to a lesser extent the biological needs of the insect (shelter, food and breeding development) could be the factors that determine the dynamic of this vector in the Ebro Delta.

Molecular study of *An. atroparvus* populations (Ebro Delta, Salamanca and Cuenca) from different places of the Iberian Peninsula has been made. New ribosomal and mitochondrial markers were developed and sequenced in all Anopheles individuals collected in prospected areas in Spain. All Culicid species were also sequenced for comparative purposes and for phylogenetic reconstructions. Genetic variability in all mosquito populations was subjected to different molecular analyses. Multigenic analyses of *An. algeriensis*, *An. cinereus hispaniola* and *An. plumbeus* from Murcia, Tenerife and Salamanca, Spain was made for first time with ITS-2, 18S and D3 of rDNA and COI, COII of mtDNA.

Capture and shipment of *An. atroparvus* females and males from the Ebro Delta to Portugal were done. This collaboration is to improve a morphometrical and molecular analysis of the different *An. atroparvus* population of Europe, in collaboration with other EDEN partners like Turkey and Italy. The degree of susceptibility of *An. atroparvus* from the Ebro Delta to the different Plasmodium species was not determined as a result of technical problems with team in charge of this test.

United Kingdom: An entomological training will be organized for the Malaria partners.

Achieved results: An introduction to the history, principles and practical applications of mosquito taxonomy, molecular systematics and the bioinformatics tools required for interpreting molecular results. Lectures on mosquito taxonomy and molecular systematics; practical exercises in molecular laboratory procedures, computer-based DNA sequence data analysis and mosquito identification; demonstrations of robotic DNA extractions, digital imaging (automontage) system and scanning electron microscopy instrumentation. Participants were provided with notebooks, handouts, lecture notes and PowerPoint presentations, computer software and literature. Mosquito specimens for molecular analysis and taxonomic study were provided by the participants and the instructors.

Outcomes: participants gained (1) an understanding of the principles of taxonomy and specimen acquisition and preservation for morphological and integrated systematics research; (2) familiarity with morphological terminology and the use of traditional identification keys; (3) practical experience of molecular techniques including DNA extractions, PCR and gel electrophoresis; and (4) ability to manipulate DNA sequence data, conduct database searches and retrieve sequences, perform sequence analysis and alignment, obtain basic sequence statistics, and generate and interpret phylogenetic trees.

Results reported from the other MAL countries:

Portugal: A total of 489 indoor resting adult *Anopheles atroparvus* were collected in the dry season (July 2008) in five locations in Portugal. With the exception of one locality in Portugal where only 17 specimens were captured, a random subset of 45 samples from each place will be used for both morphometric analysis and population genetic studies.

Ribosomic DNA was extracted from single mosquitoes and the species identification was performed following the established PCR-RFLP protocol. All the specimens were identified as *Anopheles atroparvus* and they were used for microsatellite analysis.

Genotypes of a total of 675 individuals from collections carried in Portugal (1993-1994 and 2007-2008), Spain (2008), Italy (2007) and France (2008) of *Anopheles atroparvus* will be determined for the 10 microsatellite loci selected and optimized last year from Weill et al. (2003) procedures. Specimens are currently being analysed according to PCR amplification and genotyping protocols optimized last year. Subset samples of a maximum of 45 specimens captured in the 5 localities of Portugal and in Italy, Spain and France will be sent to Turkey to be used for morphometric analysis of mosquito wings.

Morocco: A total of 4122 adult mosquitoes were collected from May to November in three locations in Morocco. They are represented by three genera: Anopheles, Culex and Aedes. Anopheles genus is represented by *An. maculupennis s.l.* (1661 specimens) and *An. cinereus* (16 specimens).

235 *An maculipennis*, 3 *An cinereus* and 371 culicinae were collected during larval investigations.

Human baiting rate, parity rate, human blood index and length of gonotrophic cycle were estimate from field and laboratory data in order to estimate vectorial capacity of *An labranchiae* for *P. Vivax*. The different vectorial capacities were shown in the following table MAL01 below.

Site	date	ma	Parity rate	Daily survivorship	Vectorial capacity
Beggara (marshy zone)	17 June	32.25	0.63	0.91	0.57
	10 July	52.00	0.85	0.97	5.85
Rouissia (lagoon)	28 July	2.00	0.87	0.97	0.28
	23 Sept.	1.00	0.75	0.94	0.041
Zhiligua (small dam)	27 may	3.00	0.67	0.92	0.012
	29 Aug.	0.5	0.87	0.97	0.066

Tab.MAL01 - An. labranchiae Vectorial Capacity, 2008

Romania: The mosquito fauna continued to have a generally extremely low abundance over all the country in 2008 because of the climate characteristics in the last three years in Romania.

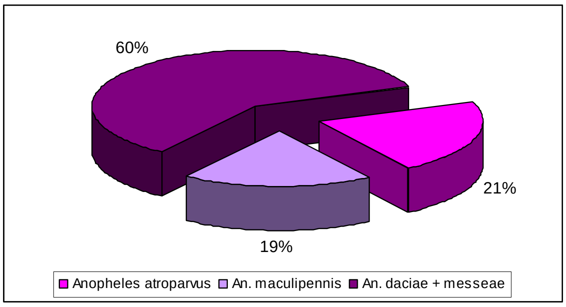


Fig. MAL03 – Proportion of the species in maculipennis group, Budeni – Comana (April – Sept. 2008)

Investigations on the anopheline fauna in Comana area in 2008: The periodical investigations of Comana lake and the area having usually temporary breeding sites in the plain near Budeni village didn't put in evidence anopheline larvae excepting a single collection of 6 larvae in June near Budeni village. The lake continued to remain with restricted surface as in 2007 and the temporary breeding sites on its borders and on the plain were missing because of the lack of rains in July and especially in August and September.

Regular collections, from February to September 2008, in animal stables in Budeni village for investigation of the species composition and dynamics of anopheline populations were performed. 1685 specimen anophelines of maculipennis group and 22 *Anopheles claviger* were collected in animal shelters in Budeni village in April, May and June.

Regular outside investigations and collections of mosquitoes by human bait, CDC light trap and bird baited trap were performed in May – August in three sites: Comana forest, on the border of Comana lake and in a courtyard in Budeni village. The collections included 375 mosquitoes belonged to 15 species (*Anopheles maculipennis s.l.*, *An. claviger*, *An. plumbeus*, *Aedes vexans*, *Dahliana geniculata*, *Ochlerotatus annulipes, Oc. Cantans, Oc. Caspius, Oc. Dorsalis, Oc. Leucomelas, Oc. Sticticus, Culex pipiens, Cx. Torrentium, Culex territans, Coquillettidia richiardii*).

The anopheline fauna in 2008 (from April - when the first generation of the year appeared - until September) was dominated in Comana area by *Anopheles daciae* and *Anopheles messeae* species with a common proportion of 59.7 % (a little higher than in 2007). The examination of the egg batches showed that *Anopheles daciae* had a proportion of about 95.0 % and *Anopheles messeae* of 5 % in the mixed populations of the two species.

After the increase of its proportion until 39.0 % in the very droughty year 2007, the proportion of *Anopheles atroparvus* decreased until 21.3 %; this value is still quite high for *An. atroparvus*, and it is in accordance with the fact that the second half of summer and September 2008 were droughty periods too.

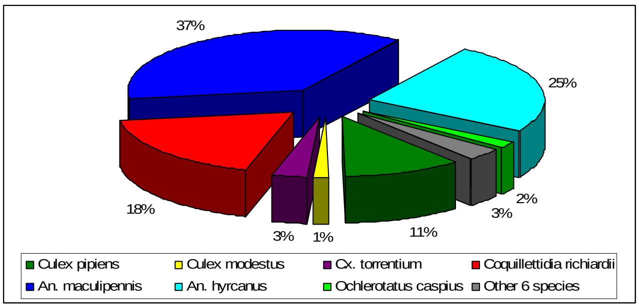


Fig.MAL04 – Mosquito species collected by light trap in Danube Delta and lagoon area – 2008

After the decrease of the proportion from 10.9 % in 2006 to only 5.0 % in 2007, the proportion of *Anopheles maculipennis s.s.* was 19.0 % in 2008; this is in accordance to the fact that the spring and June 2008 were quite rainy and colder; this species was no found anymore in July – August – September 2008 which was a droughty period with high temperatures.

The investigation of the physiological age of the anopheline females in Comana area confirmed the very important observations obtained in 2005, 2006 and 2007. The number of egg batches laid by a female shows the physiological age of the respective female and namely if the female could infect or not the humans. In the Romanian climate conditions the duration of sporogony is about 18 days; on the other hand, the anopheline female completes the gonotrophic cycle in 5 – 6 days and very rarely until about 10 days. The investigations on 319 females in 2008 (from April until September) has shown that they laid only 1 or 2 egg batches, in other words they had a very short life span, under 18 days, and few possibility to become infective for humans. These data confirm the observations made in 2005, 2006 and 2007 in this area.

Investigation of intraspecific variability of natural populations of *An. hyrcanus* by morphometric analysis of wings: Turkey has performed a morphometric study for better understanding of the morphological differentiation of *An. hyrcanus* populations from France, Czechland, Romania and Turkey. Wings of *An. hyrcanus* females were analysed (21 specimens collected in the Danube Delta).

Investigation on the anopheline fauna in the Danube Delta and lagoon area: Regular investigations and collections (February – September 2008) for the species composition and dynamics of anopheline populations were performed in Grindul Lupilor, Salcioara, Mila 26 and in other several locations (Channel and Grind Stipoc, Raducu and Rotundu Lakes, Crisan and Ghermandi Channels) in the Danube Delta and lagoon area.

The mosquitoes were collected outside on human baits and by bird baited traps, CDC light traps, entomological net (from resting places in vegetation) and inside the animal shelters, chicken coops and houses with aspirators. There were identified 17 mosquito species this year in the Danube Delta and lagoons (Fig.MAL04). The fauna has been dominated equally by An. maculipennis s.l. and *Cx. pipiens*.

Mosquitoes of maculipennis group dominated as usually the mosquito fauna in the Delta and the identified species were *An. atroparvus* (2.7 %) and *An. messeae* (97.3 %). *Anopheles maculipennis s.s.* has not appeared as usually in this area. The observation of the egg batches of the latter species showed that more than 90 % of the specimens considered *An. messeae* could be in fact *An. daciae*. The molecular investigation on these mosquitoes by DNA sequencing will clear this. *An. daciae* was recorded south from lagoon area in Dobrogea.

An. sacharovi was not recorded in the Danube Delta and lagoon area where this species had its distribution area in Romania before the malaria eradication.

Italy: Experimental infection: the second year attempts of artificial infection with a long established strain of *Plasmodium falciparum* (NF54) to estimate vector competence of *An. labranchiae* were carried out. On September 2008, during three surveys, about 1500 *An. labranchiae* field females were collected in Principina site (Maremma Region, Grosseto). Three infection experiments were performed at the Institut Pasteur by Dr C. Bourgouin, Centre de Production et d'Infection des Anophèles (CEPIA) and Dr I. Larget-Thiery (PhD). The mean prevalence of *An. labranchiae* in the 3 infection tests is 29.3%, with a very low oocysts number, (0.4% oocysts/mosquitoes). The controls, *An. gambiae* from Yaoundé, Cameroon, have shown a mean prevalence of 85.5% with a parasite burden of 24.1 oocysts/mosquitoes. There was also a 4th tentative of infection in *An. labranchiae* F1 insectary colony, reared in the Institut Pasteur. About 60 mosquitoes were infected but none has developed oocysts (prevalence 0.0%). The results of these experiments, as well as infection tests performed at the Medical Centre of Nijmegen University in 2007, shown a very low receptivity of *An. labranchiae* from Maremma, Italy to the tropical strain of *P. falciparum*, at least at the laboratory conditions.

It should be stressed that, because in 2008 the Dutch partner of Medical Centre of Nijmegen University (Dr A. Luty) was not able to do this work, we continued the study of experimental infection whith the collaboration of Dr. C. Bourgouin, (CEPIA) Institut Pasteur, Paris. **The costs of these bioassays have been supported by every Eden partner (not by the sub contract)**.

Deliverables

D MAL05 – Update of past and current Anopheline species in selected model areas and in every partner country (M48). Finished

D MAL06 – Update of the database on Anopheline collections in model areas (M48). Every partner has it own database. Data must be transfer to Management team

D MAL07 – Report and scientific articles in international journals on biology and vectorial role of Anopheline populations from model areas, (M48). Several articles already published (see list)

D MAL08 – Report on Anopheline systematics (M48) and population genetics (M48). Almost finished. Paper on *An. labranchiae*, *An. atroparvus* and *An. hyrcanus* has to be finalized and submitted by the end on 2009.

D MAL09 – Report on vector competence of mosquito populations and species not yet tested (M30 and M48). Must be done for mosquito populations and species from Spain, Algeria and Romania, and more population from Corsica, in collaboration with our new partner (Institut Pasteur Paris). An article must be written.

Milestones and expected result

M MAL08 – Collected raw data (including archived data and fieldwork in spring-summer 2005 to 2007) to create Anopheline data layers (for partners who have not finalized yet) (M48). finished

M MAL09 – Spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the model areas (M48). No major progress since last year : Completed in first model area in France, Algeria, Portugal; almost finished in first model area in Turkey; Morocco, Italy, on going in Romania, Spain; Ongoing in second model area in all country where such an area is selected

M MAL19 – Data on the biology and the distribution of *An. plumbeus* (M42). Only France has collected data. Very few specimens (larvae) have been collected. No more investigation is planned on this time consuming subject.

M MAL20 – Infection rate of natural anopheline population in Model areas in Algeria, Morocco and Turkey (M48). Done in Algeria and Morocco (all mosquitoes negative). Planned in Turkey

M MAL21 – Vector competence for populations of Anophelines in countries not tested yet (M 48). Spain, Algeria and Romania, and more populations from Corsica,

M MAL22 – First results on population genetics of *An. maculipennis s.l.* (M48). Mosquito already processed. Articles need to be written

M MAL23 – Update of distribution of Anopheline species in partner countries (M48). In progress.

M MAL 26 - First scientific publications on mosquito biology in international journal (M48) : done

WP MAL 3 – Public health and human activities

Work package number	MAL3	Start date or starting event:			37	
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	4	1	6	4	4	3	6
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	0	6	6				40

Objectives

The general objective of this WP is to understand area-wide historical malaria incidence and risk patterns in relationship with recorded public health activities and surveillance, warning and control campaigns and changing human movements patterns and activities. specific objectives of the next 18 months period are:

To evaluate the impact of public health activities, human movements, and human activities, on malaria incidence and risk.

To record and analyse past and present data on malaria incidence and the impacts of surveillance, warning and control campaigns.

Work performed during previous reporting periods

A review of literature and historical reports from Ministries of Health, research institutes and malaria control agencies has been realized in every country to obtain historical data on malaria in Maghreb and Europe (this have been done from year 1 to 3). Collection of recent data on malaria incidence (autochthonous and imported) in Maghreb and Europe has been already obtained or initiated in every country (this have been done from year 1 to 3, it an ongoing process). These data suggest that imported malaria might be underestimated in several countries. Public health activities related to malaria have been listed in every country, and their impact on malaria incidence will be evaluated in model areas where malaria used to occur. Studies on the impact of past and current human activities (modifications of agricultural and / or sociological practices, land use change; irrigation; deforestation; dams; urbanization; cattle breeding; etc) has been conducted in Camargue (France) and Morocco.

Description of work

A great improvement in the recording of imported malaria data and in human behaviour related to mosquito data has been made in almost all countries during 2007. However this study will be pursued with new surveys and in-country collaborations.

Algeria: Investigations and interviews close to the immigrant population, students, and travellers will be carried out in the second model area (El Tarf) in order to evaluate at risk human behaviours for transmission and diffusion of the disease in this model region.

Achieved results: Investigations and interviews close to the immigrant population, students, and travellers have been carried out in Ourgla, and will be carried out in the second model area (El Tarf) in order to evaluate at risk human behaviours for transmission and diffusion of the disease in this model region.

All data concerning interviews and questionnaires carried out at Ouargla among the population were compiled and analyzed. Data on autochthonous and imported malaria cases have been collected. A malaria outbreak has been detected and follow up in 2007 -08 in the South of Algeria in Tinzaouatine, causing 27 *Plasmodium falciparum* cases. Three new autochthonous cases (one infant and two adults) were detected in October 2008. The genotyping of *Pl falciparum* strains collected last year from patients in Tinzaouatine showed a resistant profile to Chloroquine. An article "Malaria in the Algerian Sahara" was finalized for submission.

France: The data obtained by questionnaires and mapping in 2007 will be submitted for publications.

Achieved results: This work is in progress.

Spain: Study of the human distribution related to biting exposure in the studied areas (Ebro Delta, Albufera of Valencia and Murcia region) in order to determine potential transmission areas. Characterization (qualitative and quantitative) of human population migrations in Andalucia and Extremadura provinces. Bibliographical review of the Malaria historical situation in Andalucia and Extremadura. Analysis of data of Malaria imported cases in Andalucia and Extremadura.

Achieved results: Human population characterization of the Ebro Delta was made following an adaptation of the French social questionnaire to carry on the same kind of study. The size of the sample according to the number of inhabitants and the risk of contact with the Anopheles populations was selected. In order to analyze the behaviour of the residents 3 towns (Poble Nou, Deltebre and Sant Jaume d'Envetja) were selected with the characteristics described on the annex. A total of 219 residents from the three towns were polled. Due the great importance of tourism in the area, we decided to interview 102 tourists during July in the different hotels and camping located in the Ebro Delta area. All the data were introduced in an EXCEL data Base. The SPSS program has been used to carry on a descriptive study of the absolute and relative frequencies.

Human movements: characterization (qualitative and quantitative) of human population migration in the three studied areas (Ebro Delta, Comunidad Valenciana and Murcia Region) has been made.

Analysis of the past situation of the illness and data on Malaria incidence: bibliographical review of the Malaria historical situation, the illness in the past, measures of the public institution during XXth century and relationship between rice and the illness in the Ebro Delta, Albufera de Valencia and Murcia Region, has been undertaken.

Malaria imported cases in Tarragona, Comunidad Valenciana and Murcia region have been recorded and analyzed.

Portugal: A manuscript entitled "Malaria re-emergence in Portugal: knowledge, attitudes and practice in a risk area" is being prepared to publication in "The European Journal of Public Health".

Results obtained in other MAL countries:

Romania: The data about malaria imported cases in Romania from the District Hospital of Infectious Diseases (Constanta, Timisoara, Iasi, Bucharest – where the patients with malaria are hospitalized) are in progress to be collected because the official data of the Ministry of Health are undercoated.

The collection of data regarding the risk of human exposure to biting by anophelines continues in Comana area and in the Danube Delta and lagoon area. In this latter area there are numerous conditions for human exposure because of the higher exophily of the anophelines than in the rest of the country. On the other part, in the second study area there is also a greater risk of the introduction of imported Plasmodium strains than in Romanian Plain because the Constanta harbor is an entrance for the ships coming from everywhere and all the Black Sea coast including the Delta and lagoons is visited by many tourists from abroad.

Italy: Public Health and human activities: The study, started in 2007, on the characterization of human communities located in Principina, (Grosseto Province) was completed in 2008. People were interviewed about human activities, relationship among environment, mosquitoes presence, malaria, about tourist and immigration movements (especially from malaria endemic countries). Although elaboration of data (demographic characteristics of the sample and the analysis of the submitted questions) is in progress, however preliminary results of 2008 seem similar to those obtained in 2007. The knowledge of residents about malaria transmission and Anophelinae biting habits appears quite good. They adopt several protective measures against mosquito bites indoor. On the contrary they didn't protect enough themselves when they are working outdoor at dusk and/or during the night. Tourists result the "at risk" group in the hypothesis of malaria re-emergence in the area. Public Health activities should be addressed to control the densities of mosquitoes and the introduction of potential gametocyte carriers in the area (e.g. illegal immigrants employed as seasonal workers in agriculture).

Deliverables
D MAL10 – Report on current and past autochthonous and imported malaria in all 8 "field" countries (M12 to M48) All partners have worked on. This D. is completed
D MAL11 – Report on human movements in relation to malaria importation/exportation in all 8 "field"

countries, and particularly in model areas (M18 to M48). Several partners remain late, however great improvement in 2008. D MAL12 – Report on the relationship between modifications of environment/human activities and malaria/potential vectors in model areas (M48). Article published for France (EID). Other partners must write reports
Milestones and expected result M MAL11 - complete data on autochthonous and imported malaria incidence including data from the model areas (M30-48): in progress M MAL12 - Complete collected raw data (including archived data and fieldwork in spring-summer 2005 to 2008) to create public health – human activities layers – particularly in malaria endemic countries) (M30-48): in progress M MAL25 – Data on human behaviour related to mosquito and malaria (M 48) : in progress

WP MAL 4 – Animal reservoirs

Not relevant

WP MAL 5 – Data management and cross disciplinary modelling

Work package number	MAL5	Start date or starting event:			37		
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	4	2	6	6	4	4	6
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	0	6	4				42

Objectives The general objective of this WP is to model the risk of malaria transmission and spread in Europe and the likely impact of environment, human behaviour and climate changes using data generated by WP1-2-3. specific objectives of the next 18 months are: - To collate climate, hosts' behaviour & environmental descriptors in relation with the distribution of malaria vectors and cases. - To model the distribution of vectors, the risk of transmission and spread

Work performed during previous reporting periods

During year 1 data has been collected, collaborators identified and trained. Workshops on modelling and data management have been organised. During year 2 and 3 data collected in WP 1, 2 and 3 have been introduced into geo-referenced databases for further analysis. Data collection including Remote sensing data and derived parameters will continue Y4.

Some first drafts of R0 models have been established by some partners (France and Turkey).

Year 3: During year 2 and 3 data collected in WP 1, 2 and 3 have been introduced into geo-referenced databases for further analysis. Data collection including Remote sensing data and derived parameters will continue Y3 and Y4. Some first drafts of R0 models have been established by the most advanced partners during year 3 (France, Turkey).

Description of work

The existing GIS capacity will be further developed in all model areas, and extended to the second model areas when applicable. R0 modeling will be initiated (or finalized) in all countries. Collaboration with HIT will continue or will be encouraged.

Algeria: To model risk factors related to the resurgence of malaria in Ouargla. To evaluate R0 in Ouragla. To collate and analyze all georeferenced data from the second model area (El Tarf). To obtain a space based cartography of vegetation, wetlands et others themes, to extract and integrate physical parameters by remote sensing techniques in the spatial database, to conduct the first analysis of the environmental indicators.

Achieved results: a model of risk factors related to the resurgence of malaria in Ouargla is under construction. The data continue to be analyzed to evaluate R_0 in Ouragla, to obtain a space based cartography of vegetation, wetlands et others themes, to extract and integrate physical parameters by remote sensing techniques in the spatial database, to conduct the first analysis of the environmental indicators. All data from different thematic layers of El KALA and Gurgle, model regions, are collected but we had problems in the GIS construction and modelling.

France: Analysis of the existing Ro model will be finalised and relationship between entomological data and human data will be analysed, and discussed regarding the malaria imported cases.

Achieved results: a first model was developed in collaboration with HIT to study relationships between landscape and density of anopheles vectors in Camargue: Tran A, Ponçon N, Toty C, Linard C, Guis H, Ferré JB, Lo Seen D, Roger F, de la Rocque S, Fontenille D, Baldet T, 2007. Use of remote sensing to map larval and adult populations of *Anopheles* species in Southern France. *International Journal of Health Geographics* 2008, 7 (9), p. 26, EDEN0059

Analysis of the risk of malaria resurgence and its potential impact was studied for the Camargue area. An R_0 model was constructed using entomologic data, malaria imported data and sociological investigations. This model was applied to future and probable scenarii to simulate the evolution of the risk of malaria resurgence. Scenarii were elaborated based on expert knowledge. They took into account climate change, land use changes, and economic context. One scenario was defined for a 25 years future and 2 others for a 50 years future. A paper has been published: Ponçon N, Tran A, Toty C, Luty A, Fontenille D, 2008. A quantitative risk assessment approach for mosquito-borne diseases: malaria re-emergence in southern France, *Malaria Journal*, 7:147 EDEN0106

An agent-based model has been developed in collaboration with the HIT Low resolution (Louvain, Belgium) for assessing malaria risk in small geographical area: Linard C, Ponçon N, Fontenille D, Lambin E, 2008. A multi-agent simulation to assess the risk of malaria re-emergence in southern France, *Ecological Modelling*, in press. Finally a R_0 mathematical model is under development in collaboration with HIT modeling team (Heesterbeek group).

Spain: Analysis of data obtained from remote sensing (NDVI, Tmax, Land use.) and their correlation with the data of population dynamics, adults and larval stages.

Achieved results: All the data needed for the development of the model have been already obtained in the Ebro Delta, with a complete GIS (with the resolution and characteristics established by the horizontal teams), entomological studies have been finished (determination of the vector capacity, dynamic and characterization of the breeding sites) and the characterization the population, migration and imported Malaria cases in the Ebro Delta. Preliminary results indicate that July and September would be the months with the greatest risk according to the human capture, *An. atroparvus* females density (good climate conditions and breeding sites with best conditions), and the longevity and length of the gonotrophic and trophogonic cycles. The period of more risk during a day according to the human capture assay data and the results of the social study (jobs and spare time activities) would be at dusk. Usually, the inhabitants of the Ebro Delta know when the mosquitoes appear and a high percentage of people use protection but few ones know that mosquito could transmit some illness.

Turkey: To model risk factors related to the resurgence of malaria in Sanliurfa, and evaluation of R0 in several locations based on recent historical data.

Achieved results: Work in progress.

Results reported from other MAL countries:

Portugal: Update of geo-referenced databases has been continued and several maps regarding climate, environment and human population parameters have been developed (see Figs. MAL05a-b). At this point we achieved a model for the *An. atroparvus* density in Portugal mainland regarding the data (presences) collection and the environmental factors.

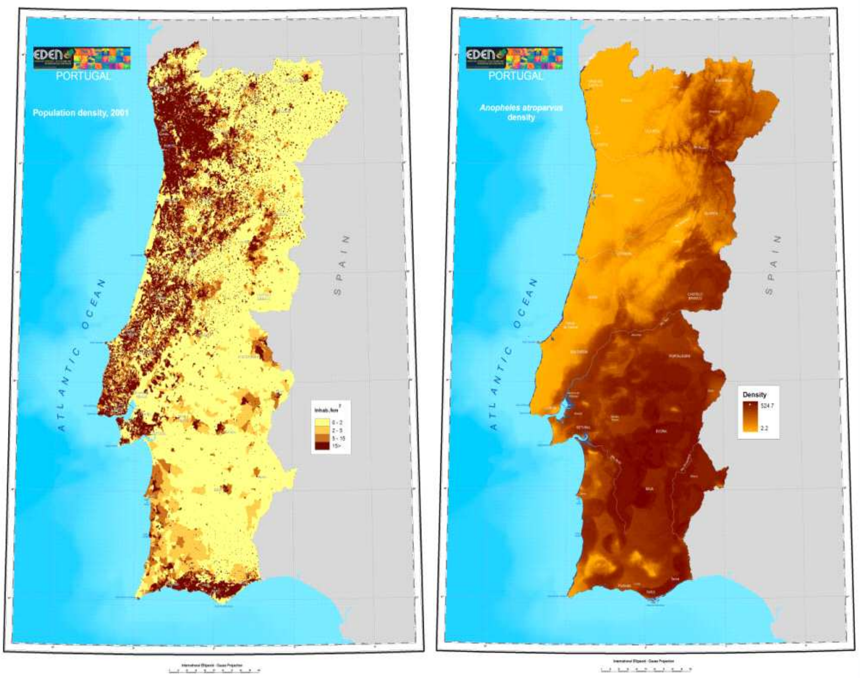


Fig. MAL05a – Left: Human population density ; Right: modelled *An. atroparvus* density

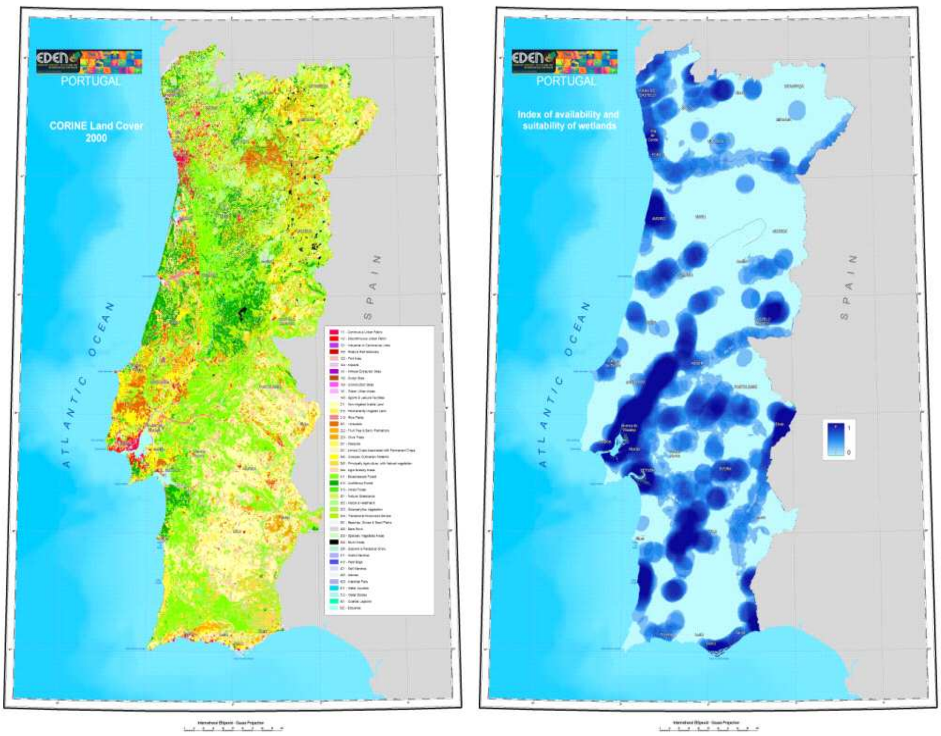


Fig. MAL05b – Left: Corine LC classes ; Right: wetlands index

To take the next step and model the susceptibility and transmission risk, the critical areas and the transmission spatial distribution and expansion, the database was enlarged with some new variables, mainly: road network and accessibility, population density, hospitals and clinics, reported cases of malaria and, house-work-house or house-school-house population movements.

Next month's we will accomplish the vector capacity, susceptibility and transmission risk maps. Then we should be able to proceed with cellular automata and multi agent simulation of malaria spreading scenarios.

Romania: The analysis and evaluation of historical and present data for collaboration with Horizontal Teams and for publication is in progress.

Italy: Data management: The distribution of potential malaria vectors over the study area, through a GIS framework, is in progress. A prediction model (10 km) and a dynamic and statistic model on the presence/absence of An. maculipennis sl, on the risk of malaria reintroduction in the study area is going on.

It should be stressed that the realisation of this tool has been supported by **extra-EDEN funds** from several partners (Italy).

Deliverables

D MAL-13: Complete (or develop when not done) the first draft of geo-referenced database on environment, vectors, malaria, and public health (M30 to M48) Ongoing in all partners

D MAL-14: Complete (or develop when not done) first draft of maps and risk models in selected areas (M18 to M48) Ongoing in all partners (done in France, Algeria, Portugal)

D MAL-15: Bibliographic database on malaria and vectors in Europe (M 48), Ongoing in all partners

Milestones and expected result

M MAL13 - Spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the 8 model areas (M30).

M MAL14 - Preliminary spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the secondary model areas (M30-42).

M MAL15 - Development of a common database to all malaria SP partners (M30 to M 48).

M MAL27 - First scientific publications on modeling in international journal (M 42).

Workpackages AFR1 to AFR5: Africa platform

Deliverable review

Work package	Deliverable number	Deliverable description	Planned delivery month	Actual delivery month	CIRAD 1 France	IRD 13 France	IAV 41 Morocco	ISRA 42 Senegal	IPD 48 Senegal
WP1	D AFR01	Census of RVF outbreaks since the 80's in the region, with available information on detailed location, date, etc.	8	38				D	D
WP1	D AFR02	Description of WNF distribution in poultry and horses in Senegal River delta and valleys	18	38	E		D		D
WP1	D AFR03	Map with selected study areas	18	38	E				
WP2	D AFR04	Spatial database of environmental information available from each study area	16	38	C				
WP2	D AFR05	Analysis of mosquitoes and ectoparasites census data in relation to their potential role in RFV epidemics	18	36		C	D	D	C
WP2	D AFR06	Comparative study on the blood-feeding entomo-fauna in disease foci and in the northern potential sites	18	38		C	D	D	C
WP2	D AFR07	Rearing of first candidate mosquitoes	18	30					C
WP2	D AFR08	Report on the vectorial capacity of local and exotic species	18	40					C4
WP2	D AFR09	Description of impact of some abiotic factors on vectorial capacity	18	40					C4
WP3	D AFR10	Report on migrant and resident wild birds species in selected areas	18	38		C			
WP3	D AFR11	Report on the WNV seroprevalence in wild birds of Senegal and Morocco	18	38			D		D
WP3	D AFR12	Comparative between site study report on candidate WNF vector	18	39		C	D	C	C2
WP4	D AFR13	Study report on the links between RVF cases and foci at Senegal river	18	38	C			C	C
WP5	D AFR14	Preliminary spatial information system and protocols for data management	18	36	D				
WP5	D-AFR-15	Extension of census of RVF outbreaks since the 80th in the region (Mali, Mauritania)	30	38				D	
WP5	D-AFR-16	Landscape typology	30	39					
WP5	D-AFR-17	Description of major breeding systems associated to landscapes	30	38				D	
WP5	D-AFR-18	Report on WN infected mosquitoes and ectoparasites	30	38		C	D	C	C
WP5	D-AFR-19	Isolation of WNV strains in Africa	30	38			D		D
WP5	D-AFR-20	Virus variability between foci (spatial and temporal) for RVF and WNF	30	38					D
WP5	D-AFR-21	Description of regional (ruminants, birds) and transcontinental (birds) movements of hosts and reservoirs	30	39		C		D	
WP5	D-AFR-22	First attempts for spreading models	30	39	C				
WP5	D-AFR-23	Integration of remote sensing data for monitoring of key environmental parameters	36	30	E				

Action		Problems	
A	Preparatory phase	1	Transportation and resource problems
B	Sources identified, requests submitted	2	Authorisations and other admin issues
C	In progress	3	Meeting or workshop postponed
D	Data collected, not yet included in central data base	4	Laboratory not operational
E	Data files included in central data base	5	Diagnostic prob lem to be checked (eg. False pos/neg)

Table AFR-01. Deliverable status per AFR partner

Executive summary

The EDEN Africa platform was able to consolidate results obtained so far, e.g. the collection of field data on candidate vectors using a variety of trapping methods. This has allowed the discrimination between host-attracted mosquito species and species actually biting the hosts, using horse-baited and chicken-baited traps. Rift Valler fever (RVF) surveillance was done on the border between Senegal Mali and Mauritania. Among the 240 ruminant sera which were analysed, a single (weakly) IgM-positive serum was detected. The risk period for horse West-Nile virus (WNV) seroconversion was identified: most negative horses became positive between October and January. Analyses on wild and residents bird sera have been achieved and new results are available but not disseminated yet.

Studies on potential RVF and WNF vectors bionomics have continued. Captured mosquitoes were dispatched in monospecific samples for virus research; however, in addition to analyses previously reported, no virus was isolated from these mosquito batches.

Rearing of vector candidates *Aedes vexans* and *Culex poicilipes* is now routinely done. However, for practical and administrative reasons, the study of vector competence of these mosquitoes has been postponed until satisfactory biosafety conditions are met.

EDEN Africa platform is still facing two problems:

- difficulties between IRD (partner 13) and Institut Pasteur de Dakar (IPD, partner 48) to find an agreement as to whom hands over results on WNV analyses made by IPD on wild-bird sera collected by IRD,
- more generally, data sharing within the platform and with horizontal integration teams is not fluent enough, thus precluding adequate integrative analysis which is the objective of EDEN. Therefore, elaboration of an agreement on data sharing is the first priority of the Africa platform.

Publications

The following PhD students are currently conducting research using EDEN data:

1. N. Diouf (Ministry of Agriculture, Directorate of Veterinary Services). Epidemiological and economical risk assessement in the horse population of the Senegal River Valley: cases of West Nile fever and African horse sickness.
2. A. G. Fall (ISRA, n°42) Bio-ecology of RVF and WNF viruses in the Senegalese river delta area.
3. Fouad El Karfaz (IAV, n°41). Study on migratory birds as possible reservoir hosts of WN virus.
4. Valérie SOTI, UMR Tetis / Neventropic. Remote sensing and modeling of vector-borne diseases – Application to Rift Valley fever in Senegal.

The following scientific documents have been published by EDEN-AFR team members. Those marked by an EDEN publication number were officially validated by the EDEN Steering Committee, those marked by EDENXXX were supported at least partially by EDEN funds but were submitted for validation to the EDEN SC after publication (no EDEN number included in final paper).

Publications of EDEN-AFR (with an EDEN number)

1. V. Chevalier, P. Reynaud, T. Lefrançois, B. Durand, F. Baillon, G. Balança, N. Gaidet, B. Mondet, R. Lancelot. Predicting West Nile virus seroprevalence in wild birds in Senegal. Vector-borne and Zoonotic Diseases, accepted (EDEN0116)

2. V. Soti, A. Tran, J.S. Bailly, C. Puech, D. Lo Seen, A. Bégué. An assessment of optical Earth Observation Systems performances for detection and monitoring of water bodies in arid areas. International Journal of Remote Sensing, accepted (EDEN0124).

Publications of EDEN-AFR (EDEN number not applied for)

None.

Meetings where papers involving EDEN-AFR ideas have been presented

1. Lancelot, R., de La Rocque, S. & Chevalier, V. 2008. Bluetongue and Rift Valley fever in livestock: a climate change perspective with a special reference to Europe, the Middle East and Africa. In: Rowlinson, P., Steele, M. & Nefzaoui, A. (ed.) Proceedings of the international conference on livestock and global climate change 2008 Cambridge University Press: 87-89
2. Soti, V., Tran, A., Lo Seen, D., Begue, A. 2008. Monitoring sahelian temporary ponds using MODIS/TERRA imagery. In: Third International Conference BALWOIS, 27-30 May 2008, Ohrid - Republic of Macedonia.

3. R. Lancelot, G. Hendrickx, D. Rogers. Le projet EDEN : approche intégrée des risques d'émergence de maladies à transmission vectorielle dans les écosystèmes européens. Application possible à l'Afrique. Conférence internationale sur les évolutions démographiques et changements climatiques: impacts sur les maladies à transmission vectorielle en Afrique de l'Ouest. Ouidah (Bénin), novembre 2008.

WP AFR 1 – Landscapes, biotopes and habitats

Work package number	AFR1		Start date or starting event:		37		
Participant id		ISRA	IPD	CIRAD	IAV		Total
Person-months / participant		8	6	6	1		21

Objectives

The main objective of this WP to identify environmental parameters and events associated with documented RVF outbreaks in livestock (ISRA, CIRAD) and humans (IP) and WNV incidence in endemic areas, and select indicators for the monitoring of epidemiological processes (CIRAD) and emergence risk.

For the next 18 months, the specific objectives of this WP are:

- To finalize the review of RVF and WNF history in the endemic area;
- To integrate the regional database including RVF events and available environmental data;
- To describe the diseases patterns in the select study sites.
- To determine the factors (environment, serological, virological and animal mobility) that determine the conditions of emergence and spread of RVF in West Africa

Work performed during previous reporting periods

The main outbreaks of RVF in livestock from 1988 to now have been documented and imported in a database. The survey of cattle sentinel herds is on going and no RVF virus circulation is detected in the Senegal river basin during the present rainy season (from June to November) in Senegal. Serological results are pending. The prevalence of IgG WN specific antibodies in sampled horses from villages and markets of the Delta of Senegal River was high. Results from sentinel chicken reveal a low circulation of WNV around the national Park of Djoudj. In Morocco, Larache area, the seroprevalence reaches 57% in Equidae.

High and very high-resolution remote sensing images were used to provide ecological maps of the study sites in Senegal, the Senegal river valley (Landsat imagery) and the Ferlo region (Quickbird). Studies on the relationships between serological, entomological, wild bird data and land-cover are planned, as soon as the data are integrated in the EDEN-AFR database.

Year 3: ISRA team visited 8 sentinel herds during the first mission in early rainy season. Two hundred and thirty sera were sampled and tested for Rift Valley fever antibodies (IgG). All test results were negative. After the rainy season, 4 sentinel herds were sampled again (240 sera) and no positive result was observed. In total, 470 sera samples were thus tested and all were found negative to RVF. Therefore, for the current reporting period, no circulation of RVF virus was observed in the survey area. In addition, IPD has prepared a document summarizing previous RVF outbreaks in Senegal and neighbouring countries, as draft of a paper to be submitted for publication.

In Morocco, 240 sheep sera were sampled from 5 different regions. None tested positive for RVFV.

Sera samples were collected by IRD in migratory and local wild birds, and transmitted to IPD for the detection of WNV antibodies. The analysis process is pending. Preliminary results indicated a WNV seroprevalence of 1.4% in Barkedji, and 1.2% in the Djoudj park.

In order to set up sentinel horses to monitor West Nile virus circulation, 400 horses have been screened to identify negative individuals to be included in a follow-up survey. It turned out that most horse sera had WNV antibodies: only 35 negative individuals were identified and included in the longitudinal survey to monitor WNV circulation. This work was jointly carried out CIRAD and IPD.

IPD has conducted a phylogenetic study of the WNV strains isolated in Senegal and neighbouring countries. This study has confirmed that both WNV lineages I and II circulate in Senegal, and that lineage I shows an important genetic variability. A close relationship was found between Senegalese, Mediterranean and European isolates, thus providing further evidence that bird migrations may disseminate the WNV. Furthermore, a new lineage of WNV has been identified and its full length genome has been sequenced.

No further study was implemented to characterize landscapes and their changes (see report of the previous period).

Description of work

All primary data will be handed over to the scientist in charge of data management in the platform (CIRAD) as soon as a general consensus is found on data sharing and management. This agreement is expected in early 2008, thus allowing the joint analysis of these data.

The expected agreement within the platform between members was not yet reached. Thus, data management could not reach the anticipated performance.

During year 3, Nicolas Diouf has begun his Phd thesis work on WNF. A one-year survey on the incidence of WNF in horses will be undertaken (CIRAD and IPD), including in a serological and clinical follow-up survey, with repeated blood sampling and clinical surveillance involving private veterinarians.

Classification per age of horses included in the serological screening

Age	First sample			Second sample			Unknown age	Total
	] 5 ; 10]	[0 ; 5]	Age > 10	] 5 ; 10]	[0 ; 5]	Age > 10		
Richard-Toll	15	60	24	16	47	31	46	239
Ross-Béthio	32	89	4	6	30	13	15	189
St-Louis	13	87	13	-	-	-	29	142
Total								570

Only 38 seronegative horses were observed after two screenings: 35 horses in the first and 3 seronegatives in the second.

First results of the sentinel horses following-up (Test ELISA)

Zones	October 2007			January 2008			February 2008		
	Pos	Neg	NF	Pos	Neg	NP	Pos	Neg	NF
Richard-Toll	3	1	2	4	0	2	4	0	2
Ross-Béthio	2	9	0	4	5	2	6	4	1
St-Louis	5	16	0	2	13	6	6	13	2
Total of Negatives		26			18			17	

Pos: Positive; Neg: Negative; NF: Not found

Economic investigations

	Horse owners	Horse sellers	Acc. sellers	Total
Richard-Toll	35	-	-	35
Ross-Béthio	69	4	2	75
St-Louis	25	-	-	25
Mpal	15	7	6	28
KMS	17	-	2	19
Total	161	11	10	182

Acc.: accessory

Serological analysis of horse sera has generated interesting results in that it has showed the risk period in seroconversion for naïve harses.

Deliverables

D AFR 02 - Description of WNF distribution in horses in the Senegal River delta and valleys and Morocco (M36).

This work was completed in Morocco and in Senegal. Results have indicated a high number of horses seropositive for WNV and seroprevalence is correlated with the age of the animals, thus the older the higher seroprevalence risk. Works in Senegal have also indicated the risk period for seroconversion.

D AFR 03 - Map of selected study areas (M32).

Selected area study map have been drawn with the support of HIT LRRS / HRRS (CIRAD).

D AFR 04 - Spatial database of environmental information available from each study area (M32). To be completed for Morocco.

Abandonned for Morocco.

D AFR 16 - Landscape typology (M32). To be completed for Morocco.

Abandonned for Morocco.

D AFR 17 - Description of major breeding systems associated to landscapes (M30).

This information could not be collected for Morocco because of difficulties encountered by the partner to work with local veterinary services. Thus abandoned.

Milestones and expected result

M AFR03 - The serological analysis have been conducted and data are available for analysis of patterns (M30).

Information is available but the database needs to be cleaned.

M AFR04 - Regional database on RVF events in domestic ruminants in Mali, Mauritania and Senegal is on the EDEN Web-site (M30). Additional data from others countries (Mali and Mauritania) will be collected.

Information is available but the database needs to be cleaned.

WP AFR 2a – RVF vector bionomics and competence

Work package number	AFR2	Start date or starting event:			37		
Participant id	IPD	ISRA	IRD	IAV			Total
Person-months / participant	45	8	0	5			58

Objectives

The global objective of this WP is to document the role of local vectors that are involved in RVF transmission in epidemic areas (Senegal river basin) and the competence of widespread candidate vectors in presently disease free areas. This will enable to estimate risk of spread in case of introduction of the virus. For the next 18 months, the specifics objectives of this WP are:

- To finalize the description of arthropods populations (mosquitoes, ectoparasites) feeding on RVF sensitive vertebrate in and out epidemic area;
- To obtain other mosquitoes species bred in controlled conditions;
- To assess infective challenge and evaluate vector competence.

Work performed during previous reporting periods

In epidemic foci of RVF, the census of mosquitoes and ticks with potential role in diseases transmission is going on in different sites from contrasted ecozones in Senegal. Three sites are studied: Djoudj national park, Ross Bethio (ISRA) and Barkedji. Similar studies are implemented in Morocco. Collected mosquitoes are tested for virus isolation, and until now, no virus has been detected.

Aedes vexans, one of the RVF candidate vector has been successfully adapted to breeding conditions. Three strains of RVF Virus isolated from human, bovine and mosquitoes were selected for vector capacity trials.

Year 3: mosquito trapping has been continued in the 3 sites of the Africa platform (Barkedji, Ross Bethio and the Djoudj Park), with CDC light traps baited with CO2, goat or sheep-baited traps, and pigeon-baited traps. Different environmental units were sampled according to a study design defined together with the high-resolution remote-sensing horizontal team.

Collected mosquitoes have been identified and monospecific samples have been prepared for virological analyses. This work has been performed at ISRA, IRD, IPD, and IAV (Morocco). In addition, IPD has used active sampling methods (aspiration of engorged females) for blood meal studies.

In total, 5,056 mosquitoes belonging to 5 genera (Aedes, Mansonia, Culex, Aedomya, and Anopheles) and 14 species were sampled and pooled into 282 monospecific samples at ISRA. Five species (Culex tritaeniorhyncus, Cx poicilipes, Cx naevi and Mansonia uniformis) represented 95% of the mosquitoes.

At IPD, the figures were, with CDC traps, 14,630 mosquitoes in 7 genera and 38 species. During year three, *Aedes (Ae.) vexans* was the most abundant species (45.4%) compared to *Culex (Cx) poicilipes* which dominated in the year two. It must be outlined that these quite different species pattern between ISRA and IPD studies are related to the contrasted environments in which the two studies were carried out: Senegal River Delta for ISRA, and Ferlo Valley for IPD (Barkedji).

From the 34 pigeon-baited traps, a total of 920 mosquitoes were collected, 98.2% of which belonged to the *Culex* genus. Pigeon was the most attractive species compared to chicken, *Cx naevi* was the most abundant mosquito species at the tree canopy, while *Cx poicilipes* was more abundant at the ground level. Monospecific mosquitoes were also pooled for further analyses, as well as engorged females for blood-meal analysis.

Candidate mosquito vectors have been successfully reared. However, biosafety conditions were not sufficient to start experimental vector capacity and competence studies. This situation will improve during the next reporting period.

At IRD, the sampling was performed in the ornithological park of Djoudj, using CDC light traps baited with CO2. In total, 573 monospecific samples were prepared for further virological analysis.

In Kenitra and Larache region (Morocco), mosquito sampling was performed with CDC traps in rice fields, permanent water pools, hen houses and stables. Preliminary results have shown the importance of 3 species: Anopheles labranchiae (33.36%), Coquillettidia richiardii (19.18%) and Culex theileri (43.62%). Monospecific samples were also made for virological research.

Description of work

Lack of funds has somehow disturbed the regular monitoring of potential RVF vectors dynamics in Ross Bethio. This site will need additional monitoring of potential RFV arbovirus vectors for at least 6 more months (ISRA).

Achieved results: At ISRA, mosquito trapping has been resumed and following results have been collected:

Fig. AFR01 - Horse-baited trap

Horse-baited trap:

In September and October, 2008: 7,039 females of mosquitoes belonging to 8 species in 4 genera (*Cx neavei, Cx tritaeniorhynchus, Cx poicilipes, An pharoensis, An ziemanni, An rufipes, Ma. uniformis, Aedomyia africana*), 29 *stomoxys* and 15 tabanids. *Culex* mosquitoes represent 92.8% (6,532 mosquitoes) were trapped out of which *Cx neavei* (39% of *Cx*) and *Cx tritaeniorhynchus* (57% of *Cx*) represented the two major species. With a proportion 89.4% of engorged (6,292) females, it was inferred that most trapped mosquitoes had presumably fed on horse.

CDC CO2 baited traps:

At Raïnabé water pond: in September-October, 2008: 3,350 females and 7 males belonging to 10 species and 4 genera (*Cx neavei, Cx tritaeniorhynchus, Cx perfuscus, Cx bitaeniorhynchus, Cx poicilipes, An pharoensis, An ziemanni, An rufipes, Ma uniformis, Aedes mucidus*) were caught. The *Culex* genus represents 97% (3,250 mosquitoes) of the captured females. *Cx tritaeniorhynchus* represented 91% (2952 females) of captured C*ulex* and *Cx poicilipes* with 6% (188 females) comes in second position.

In September-October, 2008 at Grand Lampsar site (an affluent of river senegal) 12,322 females and 42 males distributed in 10 species in 5 genera (*Cx neavei, Cx tritaeniorhynchus, Cx perfuscus, Cx bitaeniorhynchus, Cx poicilipes, An pharoensis, An ziemanni, Aedomyia africana, Ma uniformis, Aedes mucidus*). Here also the mosquitoes of the C*ulex* genus represented 97% (11,945 females) of all the total mosquito population captured. The distribution within *Culex* species was 10,330 females (86.5%) for *Cx tritaeniorhynchus* the most abundant specie followed then by *Cx neavei* and *Cx poicilipes* with respectively 8.3% (996) and 5% (593) of the sampled population.

In addition, in October, comparison was also made between CDC traps placed on the ground and in the canopy of trees. From a total of 7,933 captured females 40% (3,143) were caught with CDC light trap placed on the ground and 60% (4,790) with a CDC light trap placed in the canopy. From these 4,790 mosquitoes captured in the canopy and belonging to 10 species and 5 genera (*Cx neavei, Cx tritaeniorhynchus, Cx perfuscus, Cx bitaeniorhynchus, Cx poicilipes, An. pharoensis, An. ziemanni, Aedomyia africana, Ma uniformis, Aedes mucidus*), 4,638 (97%) are *Culex. Cx tritaeniorhynchus* remains the dominant specie with 3,511 females (76 %) was followed respectively by *Cx neavei* and by *Cx poicilipes* with 630 (13.6 %) and 493 (10.6 %) females. On the ground, only 8 species belonging to 3 genera were captured (*Cx neanei, Cx tritaeniorhynchus, Cx perfuscus, Cx bitaeniorhynchus, Cx poicilipes, An. pharoensis, An. ziemanni, Ma uniformis*). Among them 96 % (3010 females) belong to the *Culex* genus with respectively *Cx tritaeniorhynchus* 87 % (2632 mosquitoes), *Cx neavei* 10 % (297 mosquitoes) and *Cx poicilipes* 3 % (73 mosquitoes).


Fig. AFR02 - Pigeon baited trap in the canopy (left) and on the ground (right)

Pigeon baited trap:

In September-October: with a great selectivity on the attracted species, 517 females and 1 male belonging to 5 species and 2 genera are more often captured by the pigeon baited trap placed at the site of Grand Lampsar both in the canopy and on the ground. The captured species are *Cx neavei, Cx tritaeniorhynchus, Cx bitaeniorhynchus, Cx poicilipes* and *Ma uniformis*. Except one engorged female, all the others are not engorged and were thus certainly in search of a host feeding. However 91% of captures (471 females) were made on the trap placed in the canopy. Also the variety of species is higher on the canopy with 5 species than on the ground with only 3 species (*Cx neavei, Cx tritaeniorhynchus* and *Ma uniformis*). With 98 % of all the captures (508 females), the C*ulex* genus represents the majority. With very closely similar proportions in the canopy and on the ground, *Cx tritaeniorhynchus* and *Cx neavei* are both the dominant species with respectively 48% (22 females) and 43% (20 females) on the ground while in the canopy these were 47% (222 females) and 48% (225 females) in the canopy.

Chicken baited traps:
With only 126 females and 17 males, the chicken baited traps show also a big selectivity on the attracted species indeed, 7 species divided into 3 genera were captured. These were *Cx neavei, Cx tritaeniorhynchus, Cx perfuscus, Cx poicilipes, An pharoensis, An ziemanni* and *Ma uniformis*. The species of the *Culex* genus represented 94 % (119 females) of the captured population. In the distribution, *Cx neavei* and *Cx tritaeniorhynchus* with respectively 52 % (66 females) and 41 % (52 females), represented the most frequent species.

Blood meals:
Engorged mosquitoes taken with CDC light traps were put in tubes individually, and store at -20°C until the identification of their blood meals by ELISA tests.

Sentinels chicken:
Two rounds of samples were done. The first one was made on J1 on day-old chicks before their transfer in the study area: 17 out of 200 chicks were randomly sampled for that purpose. The second round was made at J15 and concerned all the populations in the three sentinel chicken poultries. All these blood samples are stored presently - 20°C awaiting for their analysis.

Fig. AFR03 - One day old chicks inside poultry (left) and view of poultry-baited trap during mosquito trapping (right)

Environmental data:
The temperature and the humidity data of every captured site are recorded during the periods of capture. Rainfall data are also recorded.

First analyses have clearly indicated that it was possible to discriminate between host attracted and host biting species when using either the horse baited trap or the chicken poultry baited trap

Depending on steering committee decision, entomology work (IPD) will continue in the Djoudj to monitor dynamics of arbovirus vectors, especially ornithophilic mosquito species and other ectoparasites (ticks). Collected data will be added to those already stored in the database.

Achieved results: This question was never properly solved and the question of discussion of results between partners and their subsequent handing over is still pending.

IPD will focus on vector competence studies of the main suspected vectors for RVF virus and the impact of environmental factors on their vector competence: *Ae. vexans*, *Cx. poicilipes* and *Cx. quinquefasciatus*. The latter will be included because of its proximity to *Cx. pipiens*, the main epidemic vector of RVF responsible for domestic transmission in East Africa and Egypt.

Achieved results: The problem related to the security of IPD's insectarium remains unsolved so far. However rearing of candidate vector is routinely done and meeting of security condition will allow test on vector capacity and competence.

Deliverables D AFR 05 - Analysis of mosquito and ectoparasites census data in relation to their potential role in RFV epidemics (M42). Samples of mosquitoes and ectoparasites have been collected and organized in monospecific groups. However analyses conducted so far did not reveal a single case of infection in any such mosquito or ectoparasite group. D AFR 06 - Comparative study on the blood-feeding entomo-fauna in disease foci and in the northern potential sites (M42). Same results found in Morocco and Senegal. No infected blood-feeding entomo-fauna was found. D AFR 07 - Rearing of first candidate mosquitoes (M36). Routinely done in the IPD's insectarium D AFR-08 - Report on the vectorial capacity of local and exotic species (M42). Not yet started for Biosafety reasons. D AFR-09 - Description of the impact of some abiotic factors on the vectorial capacity (M42). Not done.
Milestones and expected result M AFR05 - The list of candidate arthropods for RVF transmission has been up-dated (M42). No case of infection in field-trapped mosquitoes was encountered as well as in Morocco and in Senegal M AFR06 - New mosquito colonies have been established (M36). All candidates vectors have been established in the IPD's insectarium. M AFR07 - First experimental mosquito infections and host inoculations have been initiated (36). Experiments are waiting for satisfactory laboratory conditions (biosafety level) before any attempt. M AFR08 - A report on vectorial capacity of candidate vector mosquitoes is available (M42). Not available.

WP AFR 2b – WNV vector bionomics and competence

Work package number	AFR3	Start date or starting event:			37		
Participant id	IPD	ISRA	IRD	IAV			Total
Person-months / participant	12	8	0	4			24

Objectives

The global objective of this WP is to document the roles of local vectors, reservoirs and hosts and their capacity to transport WNV to Europe, directly or through relay areas. For the next 18 months, the specific objectives of this WP are:

- To finalize the census and to test wild birds in WNV endemic areas of Senegal and in Morocco ;
- To complete the collection of arthropods expected to be involved as vector and/or reservoir for WNV;
- To isolate WNV strains from vectors.

Work performed during previous reporting periods

Wild birds from selected areas (Parc National des Oiseaux du Djoudj and Ferlo Valley in Senegal, Marais de Loukos in Morocco) have been censed and sampled for WN serology. The database also includes data from other sources, notably from the Natural History Museum of Paris, where are recorded more than 1,140 migratory birds from Senegal or Europe since 1950. Blood samples from wild birds will be analyzed in the coming weeks. In addition, ectoparasites have been collected on these birds and prepared for viral analysis. Candidate mosquitoes for WNV transmission have been sampled with CDC light trap or pigeon baited trap (8309 mosquitoes, 37 species). Collections of ticks are undertaken monthly (from horse and bird nests).

Year 3: on the entomology side, the same work performed in the WP AFR 2a is still valid for this WP AFR 2b ; differences will take place in the analyses made.

Description of work

List of wild and resident and migratory bird will be updated and if necessary new layers added on the map of bird census (IRD).

Achieved results: work was to be done by IRD, a partner from whom we do no longer have any information.

Lack of funds has been a shortcoming for regular monitoring of WN potential vectors dynamics in Ross Bethio and, for other reasons, in the Djoudj (IRD). These sites will need additional monitoring of potential WNF virus arbovirus vectors for at least six more months. The possible role of horses and the important role of birds in WNF transmission will be studied using pigeon and horse-baited traps in addition to CDC light traps.

Achieved results: the activities went on only in Ross Bethio where more trapping methods have given additional information on the behaviour of vector species in face of a precise given host.

For IPD, the same activity as reported in AFR 2a will be implemented with regard to rearing WNF vectors and monitoring vectorial competence.

Achieved results: same reasons given earlier remain are valid for this section.

Deliverables

D AFR 10 - Report (list and census) on the migrant and resident wild bird species in selected areas (M42).

Not done.

D AFR 11 - Report on the WNV seroprevalence in wild birds of Senegal and Morocco (M36).

A first batch of results (serological analyses performed by CIRAD) was published: Chevalier et al., EDEN0116. More results were produced by IPD. They were not released yet.

D AFR 12 - Comparison between study site reports on the candidate WNV vector mosquito species (M36).

Not yet done because of difficulties in the data management within the plateform.

D AFR 18 - Report on WN infected mosquitoes and ectoparasites (M30).

Similarity of results generated in Morocco as well as in Senegal. No infection case found in both countries.

D AFR 19 - Isolation of WNV strains in Africa (M42).

A new strain isolated and its full length genome sequenced.

Milestones and expected result

M AFR08 - Updated bird census is available (M36).

IRD is responsible for this milestone. No information is available from this partner.

M AFR11 - Serological analyses have been conducted and prevalence data in resident and migratory wild birds are available (M36). Technical problems for ELISA specific antibodies have emerged. SN may be the option.

This is now routinely done by IPD in a two step analysis first WN and Usutu not discriminated and second step discrimination between the two viruses.

M AFR12 - WN virus strains have been isolated (M42).

The full genome of this strain has now been sequenced.

WP AFR 4 – Animal reservoirs

<table>
<tr><td>Work package number</td><td colspan="2">AFR4</td><td colspan="3">Start date or starting event:</td><td colspan="2">37</td></tr>
<tr><td>Participant id</td><td>IPD</td><td>ISRA</td><td>CIRAD</td><td>IAV</td><td>IRD</td><td></td><td>Total</td></tr>
<tr><td>Person-months / participant</td><td>9</td><td>8</td><td>6</td><td>4</td><td>6</td><td></td><td>33</td></tr>
<tr><td colspan="8">Objectives
The global objective of this WP is to qualify and quantify the routes and the patterns of introduction in Europe of hosts and/or reservoirs (livestock for RVFV, wildbirds and their ectoparasites for WNV) from endemic areas, eventually through "relay ecosystems" in West Africa (Senegal) and Maghreb (oasis, coastal wetlands of Morocco). For the next 18 months, the specific objectives are:
• To finalize the identification of key determinant of local disease spreading;
• To up date the description of regional and transcontinental movements of hosts (ruminants, wild birds).</td></tr>
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Work performed during previous reporting periods

Investigations on animal mobility in the Senegal River Delta have been conducted in study sites. Questionnaires were established and tested with local veterinary agents. Investigations on the field have validated the approach. These data are currently imported in a specific database linked with spatial models already developed. For wild birds, a database on bird ringing (from the Natural History Museum of Paris) has been made available and some maps on wild bird migrations between Europe and West Africa have been drawn.

Year 3: IRD has continued to monitor wild birds to capture a wide panel of different species and prepare batches of sera samples to be analysed. Birds have been captured using Japanese nets to mesh 12, 19 and 30 mm. Nets were placed from 6.00 to 12.00 am and from 4.30 to 7.00 pm. A total of 757 sera samples were collected in Barkedji (369) and Djoudj (388). These sera samples will be analyzed by IPD virology team. Ectoparasites have also been collected on the birds before serum sampling.

Three types of surveys were performed by ISRA team to describe animal movements in the Senegalese river delta. They took place on market places, in veterinary offices and in the rural area of Ross Bethio. More than 12,000 livestock movements were recorded, of which 82% were related to small ruminants, 16.8% to cattle, 7% to camels and 0.2 to donkeys and horses.

Twenty-two temporary settlements were recorded around Ross Bethio, out of which only 7 represented 72% of the herds investigated.

In Morocco animals movements have not been studied because of difficulties met to collect the required information from the veterinary services.

Description of work

Animal movements will continue to be studied specially on the Mauritanian side and if applicable new bird species added to the map of birds' distribution already drawn.

Achieved results: The additional results collected on animal movements have not been organized in a database yet, and still remain in bulk. In Morocco, a study over 107 samples from 17 wild birds' families has shown two positive cases in birds belonging to the *Phalacrocoradae* family (*Cormorans*).

Because field activities were postponed after payment delays in 2007, further field surveys will be implemented in 2008 (ISRA) to identify and quantify animal movements and implement the database. Moreover, data analysis will be undertaken, and risk-analysis models wil be developed (CIRAD).

Achieved results: Additional field data available but the resource person in charge of the implementation of the data base has been moved by CIRAD from Senegal.

Deliverables
AFR 13 - Study report on the links between RVF cases and foci at the Senegal river scale (M36). D AFR 20 - Virus variability between foci (spatial and temporal) for RVF and WNF (M42). Work done and presented at the Brno 2008 AGM during the PhD session D AFR 21 - Description of regional (ruminants, birds) and transcontinental (birds) movements of hosts and reservoirs (M36). Ruminant movement over the Ferlo and the Senegalese river delta available. For bird mouvements, IRD had bought two "balise Argos" for the study but no results yet available to us. D AFR 22 - First attempts for spreading models (M36). Not achieved because of difficulties in sharing data and creating an Africa platform database.
Milestones and expected result M AFR13 - Potential explicative parameters of the relationship between foci in Senegal and Mauritania are identified and mapped (M36). No available information (IPD in charge of this point). M AFR14 - A dendrogram for RVFV and WNV is drawn and describes the genetic similarities between strains from various regional areas. (M36): more virus isolates are needed. Done for RVF. No available information for WNF (IPD in charge of this point). M AFR15 - Ruminants densities and movements in sub-Saharan and northern Africa are quantified (M36). This milestone was not fully achieved because difficulties of our Moroccan partner to get information from veterinary services. Work is in progress for Senegal and Mauritania.

WP AFR 5 – Data management and cross disciplinary modelling

Work package number	AFR5	Start date or starting event:			37	
Participant id		ISRA	CIRAD			Total
- **Person-months / participant**		2	4			6

Objectives
The objective of this WP is to provide, through risk analysis and modeling tools, indicators and scenarios to anticipate the risks of introduction, emergence or re-emergence pertinent for the European, regional and national warning and surveillance systems. For the next 18 month, the specific objectives of this WP are: • To finalize the multi-sources data standardization and format for latter analysis; • To test the conceptual model.

Work performed during previous reporting periods

Data from entomological/acarological surveys, movements of animals have been transferred to the database manager. They are currently being imported to the GIS-DB in which the environmental layers are already included (thematic classification of the Senegal river valley and typology of hosts habitats). The conceptual model has been established and presented to the EDEN community during the HIT workshop on modelling.

Year 3:

Birds layer

Connection between habitats map / Birds DB (collaboration between HRRS and IRD teams). The bird database (Access) has been connected to a GIS (ArcGIS) to map:

- the distribution for each species
- the number of potentially present species in the zone

- the abundance index for each species, and an average abundance index

Mosquitoes layer

Mosquito database (collaboration between IRD and ISRA teams). A mosquito database has been designed and implemented: species distribution according to habitat, as available from the map of bird habitats.

- Mosquito species present in Senegal (vectors of RVFV or WNV) (review)
- Mosquito activity (experts' opinion)
- Mosquito habitats (experts' opinion)
- The links between these different tables have been determined using experts' opinions. A first field validation will be done.

Mosquito maps

The second approach was the mapping of mosquito habitats. Two different methods have been used:

- Apply a buffer (500 m) around all water bodies and analyse the possible habitats inside these buffers: non–supervised image classification.
- Supervise the classification using entomologists' opinion, with birds' habitats as a starting point.

Description of work

The map of Senegal River delta will be improved with new data about birds. This bird layer should yet be improved by completing the birds' DB when necessary: some classes, such as sugar cane and urban zones, were not totally completed. A validation of "experts' opinion" maps will therefore be useful species by species and to confirm specific richness and abundance index.

Achieved results: The CIRAD expert responsible for this work has been moved from Dakar.

Homogenization of DB between the different sites is planned by HRRS team (Camargue / Danube / Senegal). The next step will be the field validation.

Achieved results: The CIRAD expert responsible for this work has been moved from Dakar.

Then a new layer about mosquitoes' habitat would be added (connection with WP2). Field data will confirm and complete this map. Additional field investigations about WN dynamics are ongoing to try to evaluate the incidence and the risk period of the disease. These results will allow adding a new layer of disease data on the map and subsequent spatial analyses.

Achieved results: Not yet achieved.

All data generated integrated in the local database then in the EDEN database. Modelling expert available and starting coming up with first models.

CIRAD-IRD-ISRA-IPD (AFR): modeling of the process of persistence of the WN virus in Europe

Achieved results: There are two assumptions that can explain the persistence of the WN virus in Europe (i) cryptic cycles in unknown reservoirs, arthropods, amphibians, mammals; (ii) introduction of the virus from endemic areas, such as West Africa, via birds migrations. A mathematical model has been built to simulate these processes. Three locations are considered:

– a wet African area where the presence of birds and vectors allow WNV to circulate all year long (Senegal river valley),
– a dry African area where vectors abundance is very low during the dry season, allowing WNV to circulate only during the rainy season (Ferlo area),
– an European area where WNV may be introduced by migratory birds (Var area, France).

Data used were (i) observed seroprevalence rates in wild birds in Barkedji village (Ferlo area, Senegal) and in the Senegal River Valley, as well as an estimated annual incidence rates in horses in Barkedji village (Ferlo area, Senegal) and in the Senegal River basin ; (ii) an observed incidence rate on sentinel chickens in Barkedji.; wild birds prevalence rates and horses estimated incidence rates in the Var area.

The model is a discrete time metapopulation model with a daily time step. The epidemiological system is modelled by a set of host populations Y that share during their annual life cycle a set of locations X where vector populations live. Some of the host populations are migratory and move between locations during the year.

The model is running and emergence scenarios will be assessed: (i) short or long term climatic changes may modify the migration patterns; a mutation of the WN virus may induce avian mortality and modify the risk of emergence, either in Europe or in Africa.

CIRAD-IPD (AFR): landscape and entomological variables will be analysed to study the spatial distribution of RVF vectors.

Achieved results: Contacts were taken with IPD to start an analysis on the environmental drivers of *Culex* and *Aedes* mosquito spatial distribution in the Ferlo area. Nevertheless, the formalization of the collaboration proposal is not achieved yet.

Thus, we focused this year on the analysis of environmental risk factors of West Nile infection in the Senegal River basin. Two Landsat images were process to map the land cover of relevance for host and vectors and WNV (in collaboration with CIRAD and IRD teams). The proportion of each land cover type was calculated for each of the 5 study areas where a survey on WNV seroprevalence in horses was conducted.

Serological data were analysed using a generalized linear model, with the individual serological status as the response. As the surfaces occupied by the cover types within a buffer were highly correlated, a principal component analysis (PCA) was carried out to synthesize the initial information on the landscape into independent factors, i.e. principal component (PC). These factors were included in the model as the explanatory variables. Results highlighted a significant heterogeneity of prevalence between the study sites. PC1 and PC4 were statistically linked to the serological status ($p = 8.10^{-4}$; $p = 0.04$) suggesting that the components of the first principal component - water, grassy vegetation and inundated during rainy season "tan" - were protecting factors whereas the main component of PC4 – cultivated areas such as sugar cane - was risky factors for WNV transmission.

CIRAD-ISRA-IPD (AFR): A hydrologic model using VHRRS and LRRS data will be developed to describe the dynamics of ponds in the Ferlo area. An individual-based model will be developed to describe the transmission processes of RVF.

Achieved results: We used a Quickbird image to identify the ponds, which are the breeding sites for the mosquito vectors of RVF, in our study area (13 x 13 km around the village of Barkedji, Ferlo region). Then, a "surface-level-volume" model was implemented in a GIS to model the shape of the ponds after a calibration phase using a very high spatial resolution data elevation model. Finally, a hydric model was applied to model the ponds dynamics (input: daily rainfall; outputs: Surface, Level and Volume of the ponds). We used a dataset of water level data daily collected during 2001, 2002 and 2003 rainy seasons to calibrate and validate the model. Based on this pond dynamics model, a model of population dynamics of *Aedes* and *Culex* mosquitoes was developed. Quality assessment of the model will be achieved using entomological data from a previous project (2002 data).

On the other hand, the dynamics of host for RVF was modelled using the available information on herds and pasture management (bibliographic synthesis, expert opinion, field data) using GIS functionalities. The validation of the model and the integration between vector and host models are on-going.

CIRAD-ISRA (AFR): animal movements modelisation and related with RVF outbreaks data to explain the dynamic patterns in the emergence of the disease.

Achieved results: Trade patterns of animal movements in a specific context such as pastoral areas with nomadic pastors (Senegal valley river) are complex and difficult to study because there are many stakeholders, different kind of specific localisation (markets, veterinary post, camps) that are heterogeneously spread over the region, and a highly dynamic flow of animals exists among them. Aggregated movement data do not take into account the relative position of the units within a higher-level structure. Social network analysis (SNA) and graph theory provide a tool to organise and analyse relational data overcoming the limitations of standard methods where the position of individuals/observations does not affect the result of the analysis.
Some recorded movements of animals during the years 2006-2007, were analysed descriptively using SNA. With the data available, a directed dichotomized network with nodes and arches was realised. Relations (relative betweeness, k-neighbours and structural equivalence) between nodes were analysed in association with prevalence data. Dynamic of the outbreaks were related to the movement patterns. This approach

allowed visualization and analyses of different levels of organization that existed. Creation of a path for potential transfer of pathogens could be stemmed from this movement network.

Deliverables D AFR-23 - Integration of remote sensing data for monitoring of key environmental parameters (M30). Not yet achieved. D AFR 24 – Importation of the available field data and remote sensing data (M30). A PhD thesis has begun in 2007 (V Soti), in collaboration between CIRAD and an SME called Neventropic (applications of remote sensing). By now, the work mostly relies on pre-EDEN data because EDEN mosquito / virus data are not available. The goal is to model the spread of RVF in the area of Barkedji, using an agent-based model. The first part of the thesis was dedicated on finding remotely-sensed environmental indicators of mosquito abundance. A paper was submitted (V. Soti et al., EDEN0124). D AFR 25 – First reports of simulations in the conceptual models (M42). A PhD thesis has begun in 2007 (V Soti), in collaboration between CIRAD and an SME called Neventropic (applications of remote sensing). By now, the work mostly relies on pre-EDEN data because EDEN mosquito / virus data are not available. The goal is to model the spread of RVF in the area of Barkedji, using an agent-based model. The conceptual model was built and the computerizd model should be implemented within the end of 2009.
Milestones and expected result M AFR18. The GIS is developed and include the metadata of the conceptual model (M30). Not achieved, in progress: it will be used in the PhD thesis of V Soti.

Workpackages horizontal integration and management

WP 6.1 Horizontal data management

Deliverable review

The proposed future deliverables have remained largely unchanged despite the difficulties caused by finding delays. Though these have had a financial impact, temporary shortfalls have been met by the Partners own resources, and have not been allowed to prejudice performance or outputs.

There have, however, been some changes in strategic direction – resulting from the lessons learned from the Sub-Projects. Levels of demand for the use of the data site for data sharing and for the provision of detailed training in GIS have been low. Whilst it is possible that both will increase over the coming year, it seems unlikely. The Deliverables and Milestones for these elements have been modified accordingly

As a result the DMT has changed its focus on promoting data sharing to providing enhanced data cataloguing and dissemination of EDEN partner activities and a way of promoting both EDEN and its partners. The focus on training as originally envisaged has been replaced by a using the experience gained during the project to contribute to the formation of new Epidemiological Networks at the European level, thereby ensuring the continuation of the EDEN data archive and its components into the medium and long term.

Overview

a) Maintenance and Servicing existing products and collaborators

Both the main data site and it associated EDEN PhD site continues to expand in terms of membership, search engine visibility, and use. This requires significant resources to maintain and service. A new server was purchased and installed in June 2008, and the old one reconfigured as a backup. The site is thus now both faster, and more reliable. Combined usage regularly exceeds 150 unique visitors a day which is remarkable for a specialist technical site. A high proportion of these visitors are from outside the EDEN community, which means that the site is significantly enhancing EDEN's visibility and profile to the 'outside world'. The success of the EDEN PhD Site in particular has significantly added to the training element of the DMT activities

b) Expanding provision of standardised georeferenced datasets to Data site Members

A wide range of new datasets have been provided for the users, many of which are in the throes of detailed analyses of the data collected during EDEN's early years. These include: EU wide trees species distributions (specifically sourced for ROBO and TBD subproject members); remotely sensed indices of vegetation phenology; revised Administrative Unit boundaries at several levels for all EDEN countries; Climate change projections for temperature and rainfall from a number of sources, one in collaboration with the ECDC Funded Vborne project; new high resolution land use land cover layers ; Mammal and Avian Diversity mapping tools and outputs. There are now nearly 900 datasets in 90 data groups in 20 categories.

c) Preparation of final outputs due in 2010

Subproject data continues to expand at only a modest pace – with new information for TBD and LEISH subprojects. It has become very evident that sharing raw project data (particularly disease related information) through a central site is acceptable to only the closest collaborators. As a result the emphasis of the subproject data sharing has been moved from the exchange of sensitive data between relatively few researchers to the widest possible dissemination of what data have been acquired, from where, using what methods, and by whom, alongside a summary of any results that have been published. The intention is to describe and draw attention to the researchers and their data resources, without endangering their intellectual property rights, so that potential collaborators or indeed funding bodies can identify who best to approach for certain kinds of information and expertise. This searchable, georeferenced and mapped database is being compiled as one of the main components of the EDEN Information System that is a primary output of year 5. A number of other elements are being designed and constructed:

d) Preparing for the continuation of the data archive facility beyond the end of the project

Efforts have been in train for much of 2008 to find ways to extend the life of the EDEN Datasite beyond the end of the project, so that its content continues to be available to the network of researchers that has been developed by the EDEN approach. A number of possibilities are under discussion including the adoption of the site by the European Centre of Disease Prevention and Control in Stockholm. This would include the active advisory involvement of the ECDC in determining the details of the final DMT outputs due in 12 months time

Work package number	WP6.1	Start date or starting event:				37	
Participant id	Euro-AEGIS						Total
Person-months / participant	14						14

Objectives

The general objective of the data-management work package is to develop web-based, user-friendly data management tools and information systems needed to achieve the objectives of EDEN. The specific objectives during the second 18 months period are:

1. The further development the web-based Integrated Data Exchange and Archiving System (IDEAS v2.0) termed DMT website to assist the EDEN community in developing predictive emergence and spread models including; a) a user friendly web portal providing secured access to data archive and web resources, b) the continued strengthening of the spatial data archive, and c) an EDEN PhD web section.
2. Technology transfer and training through an improved communication network within and beyond EDEN.
3. To prepare for the development of a disease information system to be developed in the last two years of the EDEN project.

Work performed in the previous reporting periods

The first three years of the EDEN project has seen the Data Management Team establish two independent websites – one a major data archive for use by all EDEN members, and the other a site requested and used by EDEN PhD students. The data website now contains a huge volume of standardised spatial data and for use in disease modelling studies, as well as a wide variety of Sub-Project specific information for use within particular Sub-Projects.

A substantial range of information has been specifically prepared by the DMT in response to SP requests, using its in house expertise in data processing and geographic analysis.

The sites are highly visible and attract an encouragingly numerous and diverse range of visitors from inside and outside EDEN. Interest from other Projects for integration and collaboration is beginning to materialise.

The complexity and extent of the established websites means that an increasing proportion of available resources are being used for site maintenance and updating and hardware may have to be upgraded to cope with the demand.

Description of work

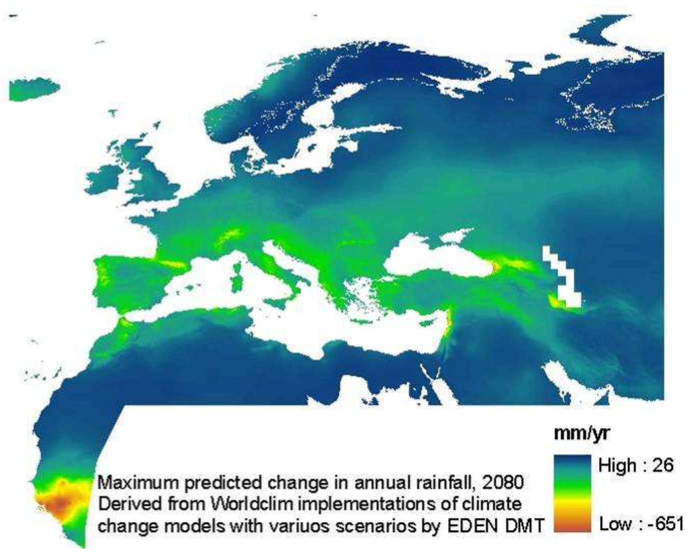
Web-based Integrated Data Exchange and Archiving System

The Team's existing achievements are such that a significant proportion of the available resources will be required to service, maintain and enhance the existing facilities – relevant data will be acquired, processed and posted as it becomes available, and additional training and advisory material will be sourced and provided for use by all EDEN partners.

Achieved results: As forecast, the increasing volumes of data contained within the data archive, the registration of new members, and the continued attention received from visitors to the site from both EDEN members and the wider global community means that the amount of time need to maintain the site and to service the membership has increased. The EDEN data archive now comprises some 900 data archives in 90 groups, divided into 24 thematic categories. This compares favourably with many of the major long established global data archives.

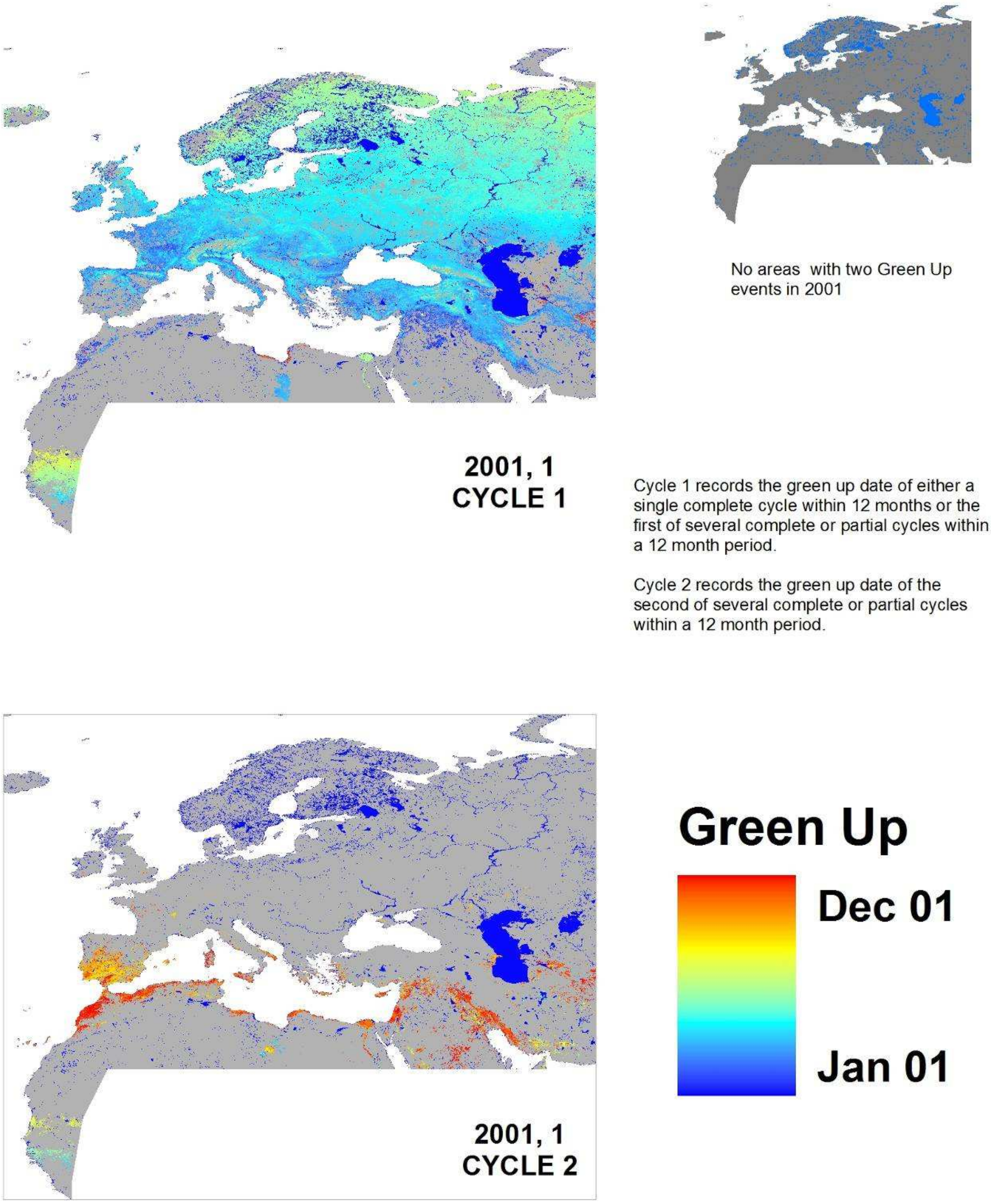
A new server and substantial extra data storage has been acquired to meet the increasing IT requirements imposed by the sites' continued expansion, and both the main site and the EDEN-PhD site have been moved to the new hardware. Most probably as a result of the public increasing profile of both the EDEN project and the Data-site itself, there have been rising numbers of unauthorized access attempts. Site security has therefore been enhanced and, to date, no such attempts have succeeded.

A substantial amount of extra data has been provided for use by EDEN members. Some of this is of a somewhat prosaic nature: for example the set of administrative boundary layers for each country in the EDEN area, at three levels of detail. These sorts of information are envisaged as useful in the report writing phases of the SP partners.



Of more scientific emphasis are the various climate change related layers that have been provided. These datasets are very substantial – in the order of 100GB, and have needed considerable time and resources to process and customize for the EDEN area. Two sources of information have been used to provide complementary layers: VBORNE - a collaborating project funded by ECDC in Stockholm; and higher resolution data derived from the Worldclim datasets held at UC Berkeley. Both consist of two primary sets of information: Baseline data for temperature and precipitation (1961-90, and 1950-2000 respectively); a series of projected climate layers as predicted by a range of global climate change models, each with a number of carbon dioxide increase scenarios, for each month for selected years in the future (see example in Fig.DMT01). These primary data have been extracted and used to produce a third dataset: the minimum, maximum and mean temperatures predicted for 2010, 2020 (VBORNE) and 2020, 2050, and 2080 (WORLDCLIM).

Other major baseline data layers provided include GLOBCOVER new 300m resolution land use/land cover from ESA, which required mosaicking from its constituent tiles; and the MODIS version 4 multiband phenology remotely sensed imagery from which the start and end of the vegetation cycles have been extracted and mapped. The figure DMT02 below shows the cycles of vegetation greening in the EDEN area during 2001



Mammal Biodiversity Mapping

The mapping of mammal biodiversity was initiated in the previous reporting period and resulted in an archive of digital presence / absence maps extracted from the Atlas of European Mammals. During this reporting period a standardised modelling protocol was developed to model these presence absence data and produce continuous Pan-European probability maps of presence/ absence using as predictor variables the data sets collated and made available through the EDEN DMT website. At the time of reporting the modelling protocol is being tested for robustness and preliminary model outputs for selected species are being discussed with specialists (see Fig. DMT03 example for *Apodemus agrarius*).

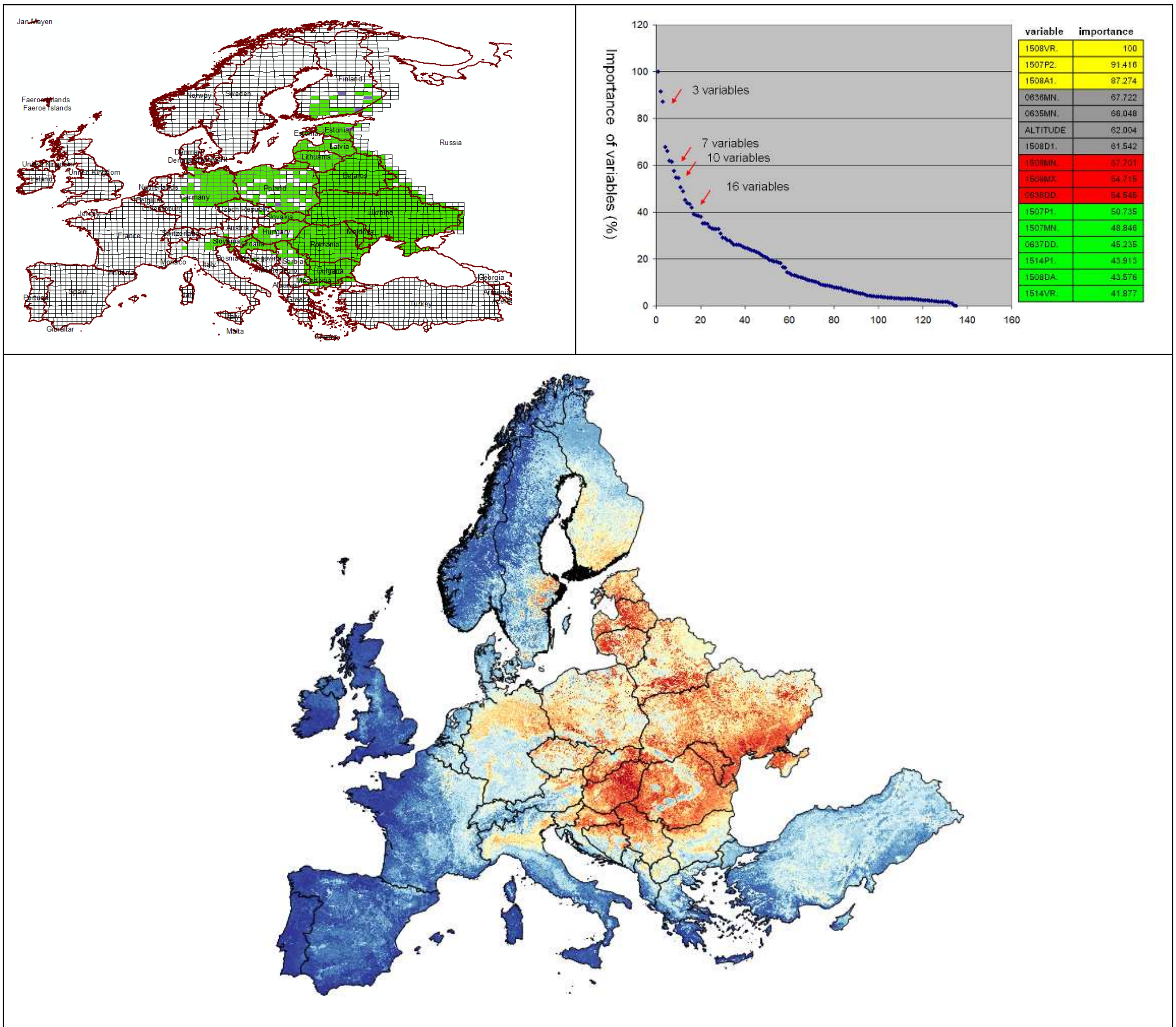


Figure DMT03 – Modelling the distribution of *Apodemus agrarius* the rodent vector of Saarema Hanta virus.

Top left map: adapted training set (green = present, white = absent) using available information on species ecology. Top right map: Relative variable importance using a random regression forest approach. Bottom map: suitability probability for Europe (red = suitable, bleu = unsuitable).

The added value of such model outputs are to (a) Identify eco-climatic variables defining species distribution limits, (b) Highlight varying suitability within the agreed distribution limits, (c) Highlight areas which are potentially suitable or unsuitable as compared to the generally accepted limits, (d) Enable to produce more detailed species and biodiversity maps to include in spatial modelling exercises of the diseases being studied by EDEN, and (e) Provide an additional data layer for spatial epidemiology studies beyond EDEN.

Mapping the potential spread of Ornithodoros sp. in Europe

As part of the V-borne project (funded by ECDC, cf. V-borne ECDC report) some preliminary work was conducted to model the potential spread of a series of disease vectors given minimum and maximum impact climate change scenarios on short and long term, using a specially developed Multi-Criteria Decision Analysis approach. Following these encouraging preliminary results, the tick expert specialised in soft ticks requested a continued technical and scientific assistance to further develop these models. Intermediary results of this work are shown in Fig.DMT04 below.

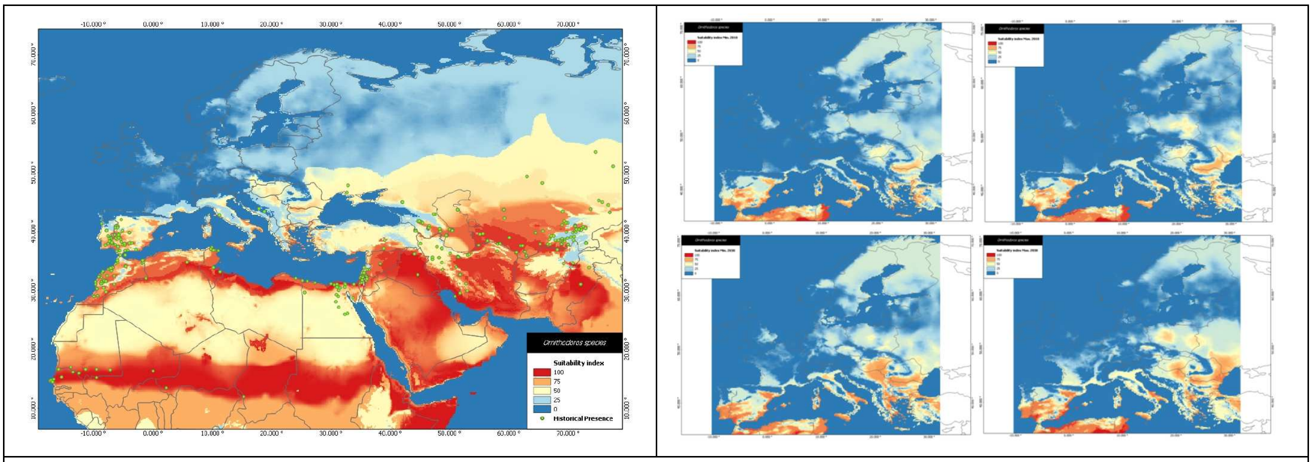
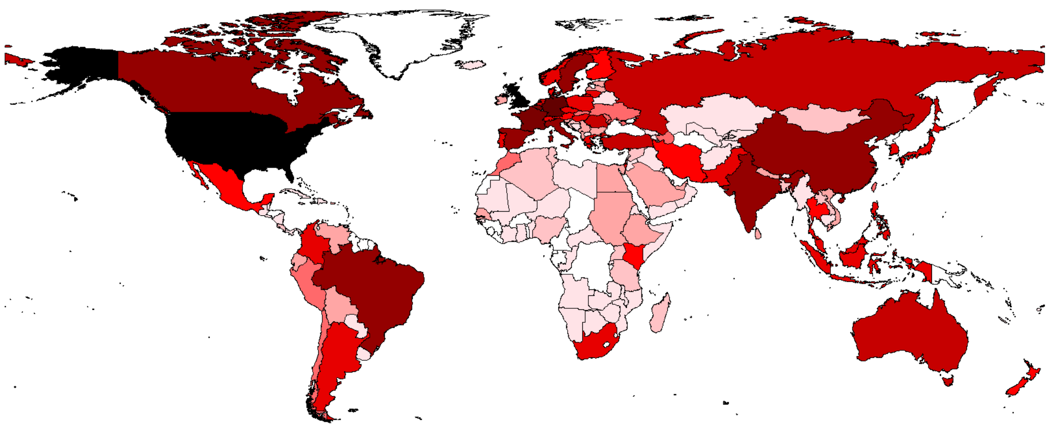


Fig.DMT04 – Suitability index for *Ornithodoros* in Europe (red = suitable, bleu = unsuitable, green dots = independent observation not used for modelling) and potential suitability shifts given different climate scenarios. Source: L. Vial, E. Ducheyne, R. Lancelot, and G. Hendrickx.

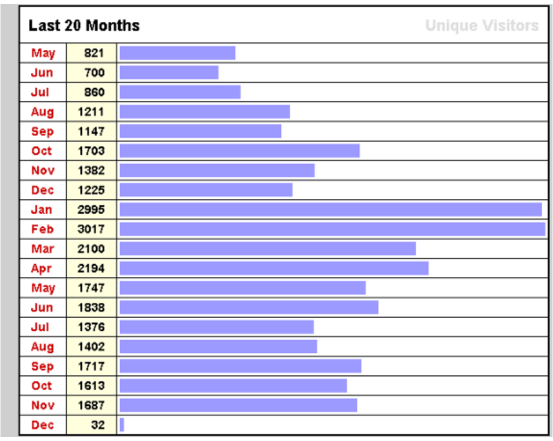
It is important to note that the MCDA method is based on expert advice on potential distribution ranges and does not rely on known presence-absence data. In the next reporting period this output will be further refined, compared to the outcome of statistical models and prepared for peer-review publication.

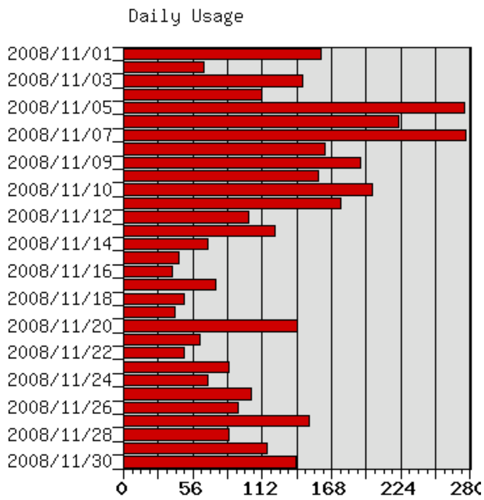
As is correctly forecasted for year three, there is a steadily rising demand for data from the SP Teams, as results from field work and data mining requires analysis in relation to external information; It is anticipated that this trend will continue, and that demand for customization of existing data, locating and standardising new data; or producing value added datasets for integration with SP projects will continue to rise. It is not, however possible to forecast what the exact demands will be, and the details will only become apparent when the requests are made.



The data site now has very nearly 300 registered users, and the number of hits to both the Data and the PhD sites continues to rise, though more slowly than in previous years. The figure below to the left show unique visitors to main site per month – peaking a about 3000 per month after the annual meeting, then averaging between 1500 and 2000 – about double the 2007 figure. There have now been a total of 38,000 unique visitors to the main site from 155 countries (see Fig.DMT05 above).

This demonstrates a consistently high and constant demand for the website content particularly as users only have to download a data set once, and thus continued demand represents new users or downloads of new data. This suggests that the sites continue to provide content that the users require after their primary data needs have been satisfied - the most popular pages remain the links and downloads pages.

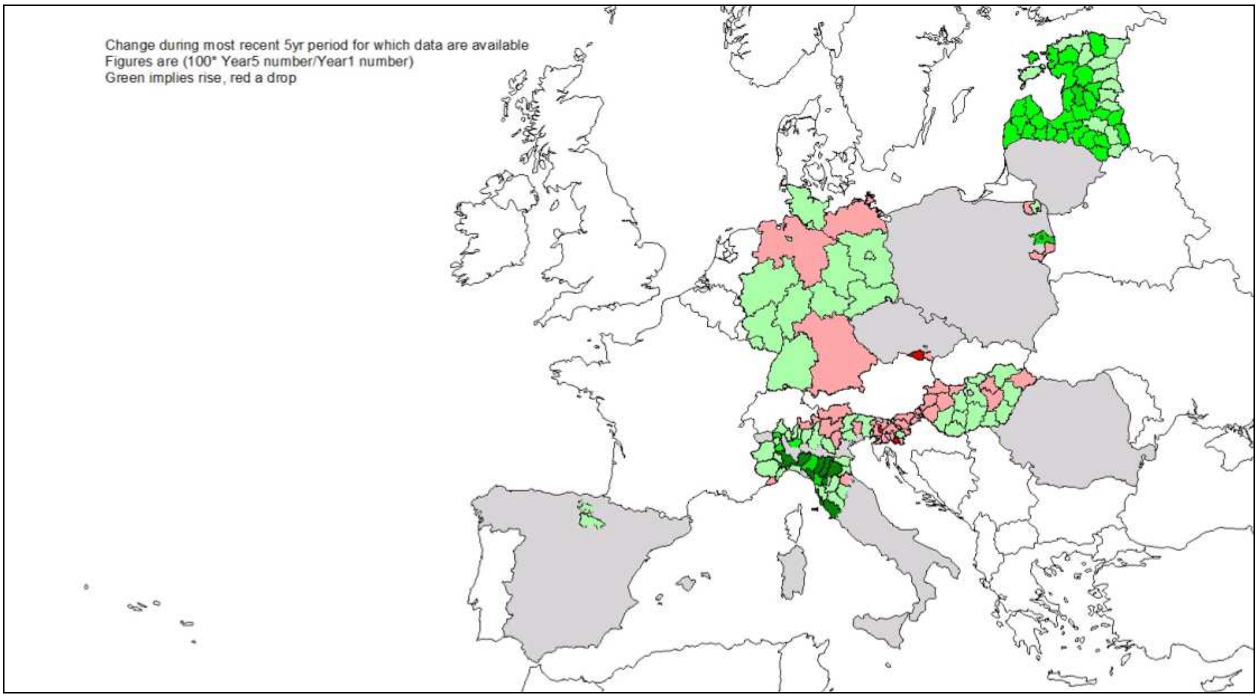




The PhD site also prospers (Fig. DMT06) - in the two years since its creation, has had 1.8million page views, and now averages 2,750 page view per day, by between 100 and 300 visitors per day. Given there are less than 100 registered users, most of these visits must be from outside EDEN, which means that the EDEN project itself is getting very significant exposure from this resource.

Specific additional secure data exchange facilities have been set up for specific collaborative analyses between the Modelling HIT and the LEISH SP, and customised data provided on demand. A number of specific data layers have been requested, - for example distributions of all the European trees species (TBD and ROBO). These have been obtained and extracted to formats more readily accessible to the research teams involved. Obtaining this particular dataset was only possible because it was requested by EDEN rather than individual researchers, and demonstrates one of the major values of the DMT concept. The acquisition of the VBORNE datasets is another of example information which would have been difficult for individual researchers to obtain.

Disappointingly, relatively few partners have proved to be willing to use the datasite to share their research data, though some information has been provided by WNV, MAL and ROBO. The TBD SP, however, continues to be the major provider of this part of the data management site, as well as requests for processing some of the data it provides, examples of which are the maps produced of the tick hosts (both wildlife and livestock) and their changes over time. And example roe-deer changes over a 5-year period is provided in Fig. DMT07 below.



During Year Four the DMT will continue to move its emphasis away from data acquisition to data provision (e.g. biodiversity models and snow cover), and it will also look for ways to capitalise on the site's established appeal to users outside the EDEN community.

The site's relatively high visibility and hit rate (number 2 for google 'europe data disease archive') provide the EDEN partners with opportunities for disseminating their EDEN material and outputs in ways not usually available to researchers. Whilst this could include both data and results, as discussed above, such a facility as not (yet) appealed to EDEN members.

As a result there has been a major shift in DMT strategy, from attempts to distribute data towards compiling and disseminating a data catalogue containing details of what data have been collected within the EDEN project, from where, by whom, and when. The concept is to produce a webpage with a searchable data catalogue, with results being both listed and mapped. This is intended not only to demonstrate the extent and diversity of the EDEN research outputs and activities, but also to serve as a means of identifying the authors and institutions most active in the topics described by the search terms used. As such it is intended to promote the partners without them needing to release their data, whilst providing the catalogue user with the details needed to initiate collaboration with the researchers identified in the search results. This concept has been used by DMT personnel in several other projects as a first stage of disseminating sensitive data, and promoting the authors, without releasing the actual data files.

This utility is now being tested prior to posting. The initial catalogue contents have been extracted from the 120 EDEN publications, from which all data collection sites, as well as the institutions involved have been located and geo-referenced. The data types underlying each publication have been categorised. The resulting database will be kept current as new EDEN papers are published, and so is likely to double or more in the coming year.

It is hoped that this 'no-risk promotion' will appeal to the EDEN community, and will lead to the active participation of research workers in providing descriptive details of their unpublished work and data holding, thereby enhancing their research profile within the search utility and so attracting more potential collaborators and funding.

The medium to long term future of the data site has also been the focus of considerable attention during the past year. Whilst it would be possible to keep the site on its current server after November 2009, there are currently no resources for maintenance and continued development. Discussions are now in progress with the ECDC to assess the possibility of incorporating the EDEN data, with all is features, into a new European Environment and Epidemiology (E3) Network. Should this initiative be successful, the continuation of the EDEN archive will be assured, one of the first instances of successful post Project funding of an EDEN product. No research data will, of course, be transferred without the express permission of the authors.

Technology transfer and training

The existing GIS training capacity will be further developed and promoted amongst EDEN partners with particular focus on EDEN PhD students.

To some degree the entire function of both sites is one of technology transfer and training. The function of main data site is to make complex spatial data more accessible to EDEN partners – it is likely that without the service much of the data would not be available to the Project as the majority of partners do not have the resources to acquire process and store much of the information.

The DMT has a number of other routine training functions which are continuously in train: the adhoc provision of advice arising from partners requests – usually in the form of emails; attendance by DMT at meetings requested by subproject partners to provide guidance in data handling, spatial analysis or GIS use (WNV, MAL); the provision descriptive help files with the more complex data sets to make them more easily accessible; the provision of links pointing to websites relevant to EDEN topics, analysis and data sources; and updating the various information pages on the EDEN PhD site (meetings, jobs etc). The popularity of the EDEN PhD site in particular (as indicated by the number of hits) has contributed significantly to the team's training output.

Specific discussions were also initiated by GIS trained personnel from the MAL SP for DMT's participation on a training course in Turkey in September 2008. DMT prepared some outlines for its contribution to the course. These, however, did not come to fruition, as the proposed course did not take place.

Epi-GIS training module

As planned the Epi-GIS training module was further developed. A set of 16 standard exercises were developed and ready to use step by step versions of each of these exercises was written for two software packages: (1) ArcGIS, a mainstream but expensive software package and (2) Manifold, a high quality lesser known but cheaper software package. The exercises are in English. A French version was also developed for the Manifold set. These exercises are now available to EDEN partners upon specific request and technical back-up is provided on-line. In the next reporting period this will be built in a proper on-line distance learning course environment using MOODLE.

Disease information system

Emphasis will be put on the transformation of the first generation data archive towards a second generation disease information system, to ensure its sustainability and availability beyond the EDEN project. Based on gained experience within and beyond EDEN and established collaborations with ECDC, ESA, DG-SANCO and GEOSS, adapted ITC tools will be identified and developed.

A framework of specific outputs for an EDEN information system has now been identified and will be presented for approval to the Scientific Committee. As discussed above the contents of the EDEN Data Site are likely to be adopted in one for or other by the ECDC, thereby ensuring the sustainability of a web based EDEN – information system in substance if not in name. A standalone DVD is also being discussed for production and distribution, containing a series of customised products as follows:

1) A subset of the EDEN data sets produced for the project (the entire EDEN data archive is much too large to fit on a DVD). This is likely to include a substantial amount remotely sensed imagery (e.g. MODIS climate and phenology), climate change assessments; biodiversity, host distributions etc. Links will also be provided to the main data site.

2) The geo-referenced searchable EDEN data catalogue described above

3) Data extract of public domain data for the known geo-referenced field sites as identified by partners and within 2) above

4) Identified, and catalogued European ecosystems and environmental conditions linked to global change – as defined by clustering current baseline conditions and using parameters from global change predictions to project into the future.

5) Published data and papers.

6) Links, tutorial and help files extracted from the Data site.

The EDEN project has requested a no cost extension until the Middle of 2010. This means that some of the final project results and publications may well not be ready before that date. It would seem inadvisable to produce the EDEN INFO system before such results are available, and it is therefore envisaged that the DVD be produced and distributed at the End Of Project conference in Marseilles in May 2010.

Whilst EDEN will most likely be capable of developing a set of tools to include in such a system, and a demo may be developed using EDEN network data, issues related to data ownership and continued epidemiological data flows will be the main bottlenecks beyond EDENs control to achieve sustainability.

Achieved results: See discussion on possible migration of site to ECDC

Deliverables

D DMT08 – Networking at international workshops and conferences to assess EDEN external user needs (continuous activity pending opportunities reported in annual report) (M36). Achieved and continuing. Attended meetings include: EMOP, Epizone, ESA risk map meetings, ESOVE, V-borne, Un Conf Poznan, WHO Conf Madrid.

D DMT09 – New epidemiological EDEN SP data sets, and new 'upon request' data sets from internal and external EDEN users are included in EDEN DMT website (continuous activity pending identified needs reported in annual report) (M36). Level of achievement: Achieved and continuing

D DMT10 – Provision of value added data processing to assist with interpretation of EDEN outputs (continuous activity pending specific requests reported in annual report) (M36). Level of achievement Achieved and continuing

D DMT11 – GIS module and exercises adapted to GIS software packages selected at AM (M30). Achieved and ongoing.

D DMT12 – Respective GIS training module manuals (M34). Achieved and ongoing.

D DMT13 – Operational PhD section (M36). Achieved and continuing.

D DMT14 – Provision of on-demand training in Geographic Analysis, using manuals, online assistance or tailored courses, as appropriate (continuous activity pending specific requests reported in annual report) (M36). Achieved.

D DMT15 - Contribution to discussion on EDEN disease information system at the EDEN SC and AG meetings, and meetings involving international user organizations. Achieved and continuing.

D DMT16 – ICT proposals for EDEN disease information system (M42). Achieved and continuing

D DMT 17 - GIS module included in Moodle distance learning tool (M60). In preparation.

Milestones and expected result

M DMT03. EDEN partners request DMT training input (M24). DMT provides inputs as required.

M DMT04. Relevant international workshops and conferences are identified to enable proper networking and identification of external user needs (M27). Achieved on a permanent basis.

M DMT07. ICT proposals for EDEN disease information system are available to start the development of disease information system tools (M42). Proposals will be discussed during AGM January 2009.

WP 6.2. RS Tools

Overview HRRS

Collaborations with several teams of vertical sub-project continued. Following these, images and data were processed and analysed. Statistical analyses were performed and models developed. Some of the results obtained have been published in joined publications.

Publications of EDEN-HRRS (with an EDEN number)

Publications of EDEN-HRRS (with an EDEN number)

LINARD, C., TERSAGO, K., LEIRS, H., LAMBIN, E.F., 2007. Environmental conditions and Puumala virus transmission in Belgium. *International Journal of Health Geographics,* 6(55). EDEN0060

TRAN, A., PONCON, N., TOTY, C., LINARD, C., GUIS, H., FERRE, J-B., LO SEEN, D., ROGER, F., DE LA ROQUE, S., FONTENILLE, D., BALDET, T., 2008. Use of remote sensing to map larval and adult populations of *Anopheles hyrcanus* (Diptera: Culicidae) a potential malaria vector in Southern France. *International Journal of Health Geographics,* 7(9). EDEN0059

LINARD, C., PONCON, N., FONTENILLE, D., LAMBIN, E., 2008. A multi-agent simulation to assess the risk of malaria re-emergence in southern France. *Ecological Modelling*, doi:10.1016/j.ecolmodel.2008.09.001. EDEN107

LINARD, C., PONCON, N., FONTENILLE, D., LAMBIN, E., 2008. Risk of malaria re-emergence in southern France: testing scenarios with a multi-agent simulation model. *EcoHealth*, submitted. EDEN1XX

PONCON, N., TRAN, A., TOTY, C., LUTY, A.J.F., FONTENILLE, D., 2008. A quantitative risk assessment approach for mosquito-borne diseases: malaria re-emergence in southern France. *Malaria Journal*, 7:147. EDEN106

Meetings where papers involving EDEN-HRRS ideas have been presented

British Ecological Society Annual Meeting, 3-5 September 2008, Imperial College, London: Lukomski, L., Vanwambeke S.O., Bennett M., Begon M., Spatial aspects of cowpox in wild rodent communities in Northern England

SOTI, V., TRAN, A., LO SEEN, D., BEGUE, A. 2008. Monitoring sahelian temporary ponds using MODIS/TERRA imagery. In: Third International Conference BALWOIS, 27-30 May 2008, Ohrid - Republic of Macedonia.

TRAN, A., PONCON, N., TOTY, C., TORRES, N., DERVIEUX, A., LINARD, C., GUIS, H., FERRE, J-B., LO SEEN, D., ROGER, F., DE LA ROQUE, S., BALDET, T., FONTENILLE, D. 2008. Application of remote sensing to assess the risk of emergence of malaria in Southern France: abstract. International Week on Space Applications, Toulouse, France, 2008/04/22-25.

Overview LRRS

Existing collaborations with various SPs continue, and new collaboration with TBD begun. LRRS data archive continues to be updated, and geo-spatial models are being developed within the existing modelling framework.

Publications of EDEN-LRRS (with an EDEN number)

SCHARLEMANN, J.P.W., BENZ, D., HAY, S.I., PURSE, B.V., TATEM, A.J., WINT, G.R.W. & ROGERS, D.J. (2008) Global data for ecological and epidemiological applications: a novel algorithm for temporal Fourier processing MODIS data. PLoS ONE, January 2008, Issue 1, e1408, 13pp. EDEN 0047.

FICHET-CALVET, E. & ROGERS, D. J (2009). Risk maps of Lassa fever in West Africa. PLoS Neglected Diseases (accepted) (EDEN ####).

PFEIFFER, D., ROBINSON, T., STEVENSON, M., STEVENS, K., ROGERS, D. & CLEMENTS, A. (2008). Spatial Analysis in Epidemiology. OUP, 142 pp. (no EDEN # possible).

Meetings where papers involving EDEN-LRRS ideas have been presented

DAVID ROGERS (2008). Using satellite and other data to make infectious disease risk maps. NaTHNaC/Hospital for Tropical Diseases Grand Round, London, January 24 2008.

DAVID ROGERS (2008). The EDEN Project. Climate-related trans-boundary pests, FAO, Rome, February 25/26 2008.

DAVID ROGERS (2008). The EDEN Project. China-EU Workshop on Energy Technology and Climate Change, Guangzhou, 6/7 March 2008.

DAVID ROGERS (2008). Here and There; now and then…… How we might incorporate Space and Time into epidemiological understanding. DIMACS/DyDAN Workshop on Climate and Disease, Ruttgers, New Jersey, April 7/8 2008.

ELISABETH FICHET-CALVET & DAVID ROGERS (2008). Spatial epidemiology of Lassa fever in West Africa. NERC Workshop, Liverpool, April 9-11 2008.

DAVID ROGERS & SIMON HAY (2008). Towards operational Risk Maps for infectious diseases. WHO, Geneva, April 14 2008.

DAVID ROGERS (2008). Climate change: a cause in search of effects? Climate, Environment and Infectious Diseases. AIBS Annual Meeting, Washington, May 12/13 2008.

DAVID ROGERS (2008). Remote sensing, geo-spatial data and disease. SPIDER Meeting, Wolfson College, Oxford, June 27 2008.

DAVID ROGERS (2008). New approaches for studying vectors and vector-borne diseases. Erice International Seminar: Medicine & Climate, Erice, August 18-26 2008.

DAVID ROGERS (2008). Environmental changes and emerging vector-borne diseases in Europe….from statistical to biological models. EMOP, Paris, August 27 2008.

DAVID ROGERS (2008). Risk mapping of vector-borne diseases – how space technologies can help. ESA Integrated Space Applications in Health, RAL, Oxford, September 3 2008.

DAVID ROGERS & TIM ROBINSON (2008). Science and Development: the bigger picture. Google Grand Challenge: Climate & Health dialogue, ILRI, Nairobi, September 9-12 2008.

DAVID ROGERS (2008). Disease and welfare mapping using satellite data. Cybersoft, Addis Ababa, September 18 2008.

DAVID ROGERS (2008). The EDEN Project: Low Resolution Remote Sensing (LRRS) Team. Joint EDEN/EPIZONE Meeting, Paris, September 24 2008.

DAVID ROGERS (2008). EDEN project and specific remote sensing needs in spatial risk modelling. ESA IAP Initiative: Disease-Risk/Disease-Vector Mapping, ESTEC, October 17 2008.

DAVID ROGERS (2008). Abiotic and biotic influences on R_0 for vector-borne diseases. R_0 and related concepts: methods and illustrations, Paris, October 29-31 2008.

DAVID ROGERS (2008). Environmental change and vector-borne diseases. Environmental and Social Ecology of Human Infectious Diseases, MRC, London, November 29 2008.

Work package number	WP6.2	Start date or starting event:				37
Participant id	ZOOX	UCL	CIRAD			Total
Person-months / participant	16	18	25			59

Objectives

The general objectives of the RS-Tools Work Package are (i) to provide the EDEN Sub-Projects with both high- and low-resolution satellite imagery products, (ii) to train and advise EDEN members on the use of satellite imagery for the monitoring of environmental changes and their application to the dynamics and distribution of vectors and vector-borne diseases and (iii) to quantify through spatial modelling the interactions between environmental changes and disease risk.

According to the Project Document (Annex 1, 6.C Milestones), the major milestones to work towards in this reporting period (Months 25 through 42) are as follows:

- M24 – First generation epidemiological models of selected diseases and pan-European eco-climatic time series analysis are available and enable the development and fine-tuning of second generation models for each of the selected EDEN diseases.
- M36 – Fine-tuned second generation epidemiological models of selected diseases are available to identify factors and indicators of change (both disease-specific and generalised) and pave the way for generic health / environment models. Results are presented at an international conference organised by EDEN.

The three HRRS HIT operational modes:

1) Service Mode, for the provision of land use and land cover and land use and land cover change maps to Sub-Projects.
2) Collaboration Mode, working with the Sub-Projects, carrying out multivariate statistical analyses linking vector density or disease incidence/prevalence to landscape scale environmental variables.
3) Land Use Research Mode, to develop new techniques for the integration of land use and spatial heterogeneity variables in epidemiology, at the landscape level.

Corresponding objectives are:

- Acquisition of high-resolution images for the study sites of the Vertical Sub-Projects and update the EDEN data website accordingly (service mode)
- Processing of high-resolution images for the study sites of the Vertical Sub-Projects, for the production of land cover/land use and land cover-/land use-change maps (service mode)
- Quantitative analyses of vector and disease database with landscape variables derived from the land cover/land use maps produced and other landscape-level data sources, with EDEN partners (collaborative and research modes)
- Production of geo-databases of vector, hosts and ecological conditions (collaborative mode)

- Agent-based modelling of human-environment interaction in the context of disease transmission (collaborative and research modes)

Specific LRRS HIT objectives for the 18 months period beginning project month 25:

Planned exchanges with EDEN partners include:

- Site visit to Senegal (Thiongane et al) (EDEN AFR)
- Site visit of EDEN ROBO (Finland, Henttonen et al) to ZOOX4
- Continued interaction with EDEN ROBO (Sweden, Olsson et al)
- Continued interaction with EDEN ROBO (Leirs et al)
- Site visit of EDEN LEISH (Ready et al) to ZOOX4 or v.v.
- Continued collaboration with EDEN TBD (Randolph et al).

The LRRS HIT operates in four modes (Project Document Annex 1, BLOCK 2 Horizontal Integration, Work Package 6.2, p. 38 – see also box below).

The four LRRS HIT operational modes:

1) Service Mode, for the provision of processed multi-temporal data to Sub-Projects.
2) Collaboration Mode, actively working with the Sub-Projects to generate statistical models of the distribution and abundance of both vectors and diseases using a variety of multivariate methods (mainly based on discriminant analytical, maximum-likelihood methods) and remote sensing data, and to produce biological or process-based models for those vectors and diseases for which sufficient data exist, or will be collected during EDEN.
3) Research Mode, to develop new techniques for the integration of the spatial information from high-resolution satellite data with the temporal information from multi-temporal satellite data.
4) Research & Service Mode, actively working with partners to develop techniques for detecting changes in habitat seasonality over time – a guide to detecting whether habitats have changed from the perspective of the vector-borne diseases within them. A secondary objective here is to establish the correlations between the old (e.g. AVHRR) and new (e.g. MODIS) satellite data time series.

Applicable to respective modes specific LRRS HIT objectives are:

- To continue to download MODIS and other data as appropriate, to establish contemporary pictures of European habitats (service mode).
- To continue to process the downloaded imagery and to update the EDEN website with processed data (service mode).
- To apply information-theoretic statistical models to the datasets provided by EDEN partners to date (collaboration mode).
- To begin to develop satellite-driven biological models for selected EDEN vectors (collaboration mode).
- To investigate with the HRRS partner 4 (UCL) the integration of low- and high-resolution imagery for selected EDEN field sites (research mode).
- To investigate continent-wide seasonality changes over time, as detected by the AVHRR time series; are they artefactual or real? (research and service modes).
- To continue capacity building and technology transfer (all modes).

Two groups contribute to the Remote Sensing (RS) Work Package Outputs: the High Resolution Remote Sensing Horizontal Integration Team (HRRS HIT) with inputs from UCL and CIRAD, and the Low Resolution Remote Sensing Team Horizontal Integration Team (LRRS HIT) with inputs from ZOOX.

Work performed by HRRS in the previous reporting periods

As part of the 'environmental change' (HRRS) HIT, we continued to work closely with all sub-projects. With each sub-project, we conducted one or several empirical data analyses on a specific disease, in a specific region or country, and on a dataset collected by sub-project partners. All these projects involved high resolution satellite imagery for land cover classification and change detection, and modelling of these data with vector, host or human data in a spatially-explicit way. We modelled vector/host presence-absence, abundance, and serological prevalence, as well as human cases of infection.

We continued collaboration with UA-ROBO to analyse spatial variations in Hantavirus infection risk in Belgium. At first, relationships between density of bank voles and Hantavirus prevalence in bank voles population and a series of landscape level environmental variables were investigated. Secondly, the link between environmental and socio-economic factors and the distribution of Hantavirus human cases in Belgium was explored.

Collaboration with MAL implements an agent-based model on the evolution of human-mosquitoes contacts according to global changes in order to assess the risk of re-emergence of malaria in Camargue (France). It also involves a study of the link between larval distribution and environmental variables.

Collaboration with the university of Liverpool on cowpox and rodent dynamics was continued.

We collaborated with TBD to study the relationships between tick-borne encephalitis and land cover and landscape structure in Latvia, first using land-cover data derived from Landsat images and socio-economic data at the parish level.

We conducted a project with the LEI team for leishamniasis in southern France, in the Pyrenees region. Based on land-cover data derived from Landsat images, we computed forest and landscape fragmentation metrics and related these to data on the trapping of sandflies. We continued collaborations with WNV teams to study the emergence factors of WNV in Southern France and the role of birds/mosquitoes species in the introduction, amplification and spread of the virus. Collaboration with the Danube Delta Institute started to produce habitats map for birds in the Danube Delta; the link between environmental variables and the equine cases in this area was explored. Collaborations with AFR-Senegal teams continued for the study of WNV in the Senegal Delta and RVF in the Ferlo area. We started to study the migration bird pathways from Africa to Europe.

We attended several meetings with vertical project colleagues (in Oxford, London, Antwerp, Tulcea, Montpellier…). Catherine Linard conducted field work in Camargue, Arnaud Roux in the Danube Delta. Eric Lambin attended the scientific committee meetings. We all attended the annual EDEN meeting. We presented our work to international scientific meetings and produced a couple of scientific publications.

Description of work – HRRS

UCL-Liverpool (ROBO): the datasets of landscape and environmental data and of rodent population and infection prevalence will be collated and analysed jointly by the two teams in the coming year.

Achieved results: a joined field visit was organized in November 2007 with the purpose of validating the land cover, to visit trapping sites of Liverpool (ROBO), and discuss relevant environmental variables for studying rodent and cowpox infection prevalence dynamics. Following this visit, it was decided to acquire SPOT-XS image data at 20 meters resolution. Because Kielder forest is a commercially exploited forest, it has a very dynamic landscape and it is necessary to use images dating from the same period as rodent trapping. A focus on environmental aspects relevant to rodent and cowpox ecology was taken when classifying the image. Further methodological aspects were discussed during a meeting in UCL in February 2008.

UCL-ZOOX (TDB): A multivariate model of landscape-related risk factors for TBE in northeastern Latvia will be produced.

Achieved results: the presence of reported symptomatic tick-borne encephalitis cases among residents of rural parishes of Latvia was investigated in close collaboration with ZOOX-TBD. Three major characteristics of parishes were investigated: whether their environment is suitable for tick populations (i.e. land cover); whether the local human population is likely to enter the forest on a regular base (i.e. land use); and whether the spatial distributions of these two aspects are likely to intersect, through access rules (land tenure). A diversity of data sources were included to describe these three aspects: Landsat-based land cover images, CORINE land cover data, Latvian household census data, Latvian agricultural census data, and Latvia State Forest Service data. Detailed land cover classifications were produced based on Landsat images from 1988, 1992, 2000 and SPOT images from 2007. Multivariate statistical models were elaborated using logistic regression models, both in a non-spatial and in an autoregressive setting. Two levels were considered: the Vidzeme region in the northeast of Latvia, and the whole country. Results indicate that all three aspects are important to understand the spatial distribution of tick-borne encephalitis presence in rural areas of Latvia; however results vary between both levels of investigation, indicating that regional variations in risk factors related to human behaviour are important. The concept of landscape is here given new depth by consideration of its physical structure, its use by human populations, and of its accessibility as modulated by tenure. Land tenure of forest in Latvia significantly influence two aspects relevant to tick-borne encephalitis transmission: access to the forest, and forest management. Local landscape conditions consist in a complex combination of land cover and land use. Moreover, how the land is managed and how it is accessed as regulated via land tenure influence the level of risk to TBE. Understanding the spatial distribution of TBE required all three aspects to be detailed. Results indicated that land cover, land use and land tenure were important for understanding the spatial distribution of TBE in Latvia. Results clued at differences in human populations, behaviour, and risk at the regional level.

UCL - NHM (LEI) - UU (MM) – ZOOX (LRRS): Using the example of sandflies and leishmaniasis, integration across horizontal teams will be investigated by the construction of biological models relying on high- and low-resolution data.

Achieved results: All tasks carried out over the latest period target the production of a high-resolution R0 map, based, among other things, on a land cover derived sandlfy abundance map. This also allows investigating sandfly habitat and ecology using high-resolution remote sensing and tools from landscape ecology, neither of which have been frequently used for this purpose. In order to produce the sandfly abundance map, sandfly trappings for 2005 (data collected by NHM-LEI) were combined to variables describing the landscape around georeferenced trapping sites in a statistical model of abundance (negative binomial regression). These results extend the work previously carried out. Buffer sizes of 1000 and 2000 meters were used. Shannon diversity index were calculated for each buffer size. *P. ariasi* abundance is well predicted using landscape variables. *P. perniciosus* seems to have more flexible habitat requirements. These results will serve as a base to extend the modelling to more trapping data and a wider diversity of landscape variables. The sandfly abundance model and map will in turn be included in the spatialisation of the mathematical model proposed by UU-MM.

UCL-SMI-(ROBO/TBD)-ZOOX (LRRS)

Achieved results: spatial data on TBE cases was transmitted by SMI to UCL for the purpose of investigating what are the landscape-level environmental characteristics of place where people acquire TBE infection. Cases are recorded by administrative place of infection. Spatial data on these administrative divisions (Smaorter and Tattorter) have been acquired and are used to relate places of infection to environmental information extracted from CORINE land cover data.

UCL-IRD (MAL): the agent-based model will be improved and will allow testing of the impact of different land-use changes scenarios on the vector-host ratio in Camargue. The agent-based modelling will be possibly applied to other locations.

Achieved results: a multi-agent simulation (MAS) was developed in collaboration with the MAL vertical team (IRD) to assess the risk of malaria re-emergence in the Camargue in southern France, a non-endemic area where mosquitoes of the genus *Anopheles* (Diptera: Culicidae) live. The contact rate between people and potential malaria vectors, or the human biting rate, is one of the key factor to predict the risk of re-emergence of malaria, would the parasite be introduced in the region. Our model (called MALCAM) represents the different agents that could influence malaria transmission in the Camargue – people, mosquitoes, animal hosts and the landscape – in a spatially-explicit environment. The model simulates spatial and temporal variations in human biting rate at the landscape scale. These variations depend on the distribution of people and potential vectors, their behaviour and their interactions. A land-use/cover map was used as a cellular-spatial support for the movements of and interactions between mobile agents. The model was tested for its sensitivity to variations in parameter values, and for the agreement between field observations and model predictions. The MALCAM model provides a tool to better understand the interactions between the multiple agents of the disease transmission system, and the land use and land cover factors that control the spatial heterogeneity in these interactions. All the suitable factors of the disease transmission system are present – competent mosquito vectors, habitats for their breeding, and susceptible people – except for the parasite. It allowed simulating the effect of changes in potential drivers of malaria re-emergence. Potential drivers were related to biological attributes of vectors, agricultural practices, land use, tourism activities and climate. Scenarios of plausible futures were formulated and then simulated using the MALCAM model. Scenarios were developed by varying the value of model inputs. Model outputs were then compared based on the contact rate between people and potential malaria vectors, and the number of new infections in case of re-introduction of the parasite in the region. Model simulations showed that the risk of malaria re-emergence is low in the Camargue. If the disease would re-emerge, it would be the result of a combination of unfavourable conditions: introduction of a large population of infectious people or mosquitoes, combined with high levels of people-vector contacts resulting from significant changes in land use, tourism activities, agricultural policies, biological evolution of mosquitoes, and climate changes. The representation in the MALCAM model of interactions and feedbacks between different agents, and between agents and their environment, led in some cases to counter-intuitive results. Results from scenario analyses can help local public health authorities in policy formulation.

CIRAD-IRD (MAL): Some land-use changes scenarios in the Camargue will also be tested to assess their impact on vector abundance / R_0 maps. It will be interesting to compare and discuss results of the two approaches: agent-based modeling and mathematical modeling.

Achieved results: in parallel to the multi-agent simulation of potential transmission of malaria in Southern France, we developed a quantitative risk assessment approach for malaria in the Camargue area, using the vectorial capacity and the R_0 as risk index for malaria emergence. The results showed a very low risk for malaria re-emergence in the whole Camargue area; the monthly maps of the *An. hyrcanus* vectorial capacity helped to highlight the areas and the periods for which the risk is maximal.

In the second step, two main 50-year changes scenarios were tested, taking into account climatic changes, urban and touristic development and economics politics (impact on rice agriculture). The changes were taken into account in the land use map, thus in entomological index and R_0 maps, using GIS modelling facilities. Both scenarios indicated a low risk in Camargue; however, the location of the areas more at risk changed (shift to the northern part of the area).

UCL-Finland (ROBO): Quickbird images will be acquired and processed in order to analyse Hantavirus prevalence in rodents in a study zone of Finland.

Achieved results: environmental variables were extracted from a subset of a Landsat 7 ETM+ image at 30 m resolution, acquired on May 29, 2002 (Path 188, Row 016, Global Land Cover Facility (GLCF), Maryland). The image was radiometrically and geometrically corrected at the EOS Data Center of the USGS and georeferenced using topographic maps of Finland. The image subset covers the study zone of Konnevesi (between 62°33 and 62°36 north, and 26°19 and 26°24 east). The Tasseled Cap transformation of Landsat ETM+ data provided three indices related to biophysical attributes of the surface: soil brightness, greenness of the vegetation, and soil and plant wetness. The Normalized Difference Vegetation Index (NDVI) was also extracted. The Landsat image subset was also classified using the standard supervised maximum likelihood method. Environmental remotely-sensed data will be linked to bank vole abundance and PUUV prevalence data collected by the ROBO team in Finland.

CIRAD-EID-IRD (WNV): the risk areas identified according to three scenarios of introduction will be compared with areas of virus circulation (equine cases, seropositive birds) during the last years. Land-use and climatic changes analysis will be performed to study the potential emergence years.

Achieved results: Using a landuse map linked to bird and mosquito abundance and diversity databases, the areas the most probable for WNV circulation was identified according to several scenarios resulting from different combinations of hypotheses concerning:

- introduction of the virus in the area: by migratory birds from Africa in spring; by migratory birds from East or Northern Europe in Summer; by overwintering mosquitoes;
- amplification of the virus: by mosquitoes (1 or 2 species) / by birds (magpies and sparrows; species listed by Jourdain et al. (2007); zones of high/low diversity; all species);
- spatial diffusion of the virus: magpies and sparrows; species listed by Jourdain et al. (2007); zones of high/low diversity; all species;
- emergence: by *Culex modestus, Culex pipiens*

From these numerous combinations, maps of potential circulation zones were mapped, highlighting strong spatial heterogeneities; they will be compared with known areas of virus circulation in the equine populations.

CIRAD-DDI (WNV): distribution maps of birds species potentially involved in the WNV transmission processes will be produced. Impact of bird diversity and abundance on the WNV circulation will be studied and results will be compared with the french area.

Achieved results: in collaboration with the Danube Delta Institute (DDI), maps of the distribution of bird species were created, based on a land cover map derived from Landsat ETM+ imagery and a Bird Database developed by the DDI. Richness and Diversity indices were also calculated. The validation of these maps is in progress. This step is necessary to go further into the analysis.

CIRAD-IPD (AFR): landscape and entomological variables will be analysed to study the spatial distribution of RVF vectors.

Achieved results: contacts were taken with the Institut Pasteur of Dakar (IPD) to start an analysis on the environmental drivers of *Culex* and *Aedes* mosquitoes spatial distribution in the Ferlo area. Nevertheless, the formalization of the collaboration proposal in not achieved yet.

Thus, we focused this year on the analysis of environmental risk factors of West Nile infection in the Senegal River basin. Two Landsat images were process to map the land cover of relevance for host and vectors and WNV (in collaboration with CIRAD and IRD teams). The proportion of each land cover type was calculated for each of the 5 study areas where a survey on WNV seroprevalence in horses was conducted.

Serological data were analysed using a generalized linear model, with the individual serological status as the response. As the surfaces occupied by the cover types within a buffer were highly correlated, a principal component analysis (PCA) was carried out to synthesize the initial information on the landscape into independent factors, i.e. principal component (PC). These factors were included in the model as the explanatory variables. Results highlighted a significant heterogeneity of prevalence between the study sites. PC1 and PC4 were statistically linked to the serological status ($p = 8.10^{-4}$; $p = 0.04$) suggesting that the components of the first principal component - water, grassy vegetation and inundated during rainy season "tan" - were protecting factors whereas the main component of PC4 – cultivated areas such as sugar cane - was risky factors for WNV transmission.

CIRAD-ISRA-IPD (AFR): A hydrologic model using VHRRS and LRRS data will be developed to describe the dynamics of ponds in the Ferlo area. An individual-based model will be developed to describe the transmission processes of RVF.

Achieved results: we used a Quickbird image to identify the ponds, which are the breeding sites for the mosquito vectors of RVF, in our study area (13 x 13 km around the village of Barkedji, Ferlo region). Then, a "Surface-Level-Volume" model was implemented in a GIS to model the shape of the ponds after a calibration phase using a very high spatial resolution DEM. Finally, a hydric model (Puech, 1992) was applied to model the ponds dynamics (input: daily rainfall; outputs: Surface, Level and Volume of the ponds). We used a dataset of water level data daily collected during 2001, 2002 and 2003 rainy seasons to calibrate and validate the model. Based on this pond dynamics model, a model of population dynamics of *Aedes* and *Culex* mosquitoes was developed. Quality assessment of the model will be achieved using entomological data from a previous project (2002 data).

On the other hand, the dynamics of host for RVF was modelled using the available information on herds and pasture management (bibliographic synthesis, expert opinion, field data) using GIS functionalities. The validation of the model and the integration between vector and host models are on-going.

Work performed by LRRS in the previous reporting periods

This team operates in 4 modes, ranging from simple (the provision of LR remotely sensed satellite data to EDEN partners) to advanced (the development of new RS approaches and algorithms for problems encountered by EDEN partners).

Our service provision of processed RS data continued this year with the completion of a series of temporal Fourier processed v4 MODIS imagery for 2001 to 2005 inclusive (processing requires entire years' worth of data so cannot be completed until after years' end) in the middle infra-red (MIR), daytime Land Surface Temperature (LST), night-time LST, Normalised Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) 'channels'. Images were re-projected from the Sinusoidal projection to the latitude/longitude system, for ease of use by EDEN partners. The LRRS team also worked with EDEN ROBO and EDEN LEISH partners to develop risk maps for Hantavirus data from Sweden (in collaboration with Gert Olsson) and for both leishmaniasis and two of its key vectors throughout France (in collaboration with Paul Ready).

We continue to develop algorithms for using disease data recorded at administrative (i.e. polygon) and completed an analysis of the long-term AVHRR time series, investigating 'significant' trends in the temporal Fourier components of LST and NDVI imagery. Related work outside of EDEN involved developing risk maps for BT and its vectors in the UK, and for Lassa fever in West Africa.

David Rogers attended the EDEN AGM, all EDEN SC meetings, went on the EDEN mission to Senegal, visited ECDC, ESA and DG-SANCO to discuss EDEN activities/future and made presentations at six international meetings at which the work of EDEN was mentioned or show-cased.

The LRRS HIT team has downloaded and processed multi-temporal satellite data, examined changes over time in the longest time series of multi-temporal data available, and developed statistical risk predictions using datasets from EDEN-ROBO, EDEN-LEI and EDEN-TBD.

Description of work – LRRS

The LRRS team furthered collaboration with the EDEN SPs to apply the statistical approaches to disease risk mapping developed at an earlier stage of EDEN. We have investigated a new idea for investigating the importance of individual variables in disease distributions using an information-theoretic approach that has so far been applied to mosquito distributions and to Lassa fever in West Africa. It is now being applied to EDEN TBD data for Hungary. During the year plans were made to integrate most of the HITs with the individual SP teams, beginning with EDEN-LEI.

Achieved results: This work really began at the end of the calendar year.

ZOOX (HIT). Continuous updating of processed MODIS data archive.

Achieved results: updating of processed MODIS data archive continues with downloading and Fourier processing of MODIS v5 data for the period 2001 - date that promises greater stability through an integration of data from both the Terra and Aqua satellites. The new data set also has, as a standard, a 500m visible channel which will therefore allow the production of vegetation indeces at this resolution. We are processing these data for the EDEN area (only) at present.

ZOOX (HIT)-ROBO (Finland/Sweden). Development of hanta risk maps for Finland/Sweden.

Achieved results: collaboration continued during the year using datasets kindly provided by Gert Olsson of the ROBO team on hantavirus, tularemia and TBE in Sweden. Because of the accuracy of case geo-location, and the more or less complete coverage (i.e. virtually all cases nation-wide are reported), we should be able to explore methods for risk mapping when data sources are good, and therefore we should also be able to appreciate the reasons for false negative and false positive predictions. Field work is being carried out, based on the risk maps produced to date, to investigate such model inaccuracies in targeted places within Sweden.

ZOOX (HIT)-ROBO (Belgium). Development of hanta risk maps for Belgium/France/Slovenia.

Achieved results: this work was delayed for lack of a post-doc to fill the EDEN position on the team. Now that the post is filled, we should be able to complete shortly.

ZOOX (HIT)-ZOOX(TBD). MODIS-based TBE- and other risk maps.

Achieved results: work on a valuable Hungarian TBE dataset from EDEN TBD is producing risk maps for this country spanning the period from the 1970s to date. Over this time TBE has changed its spatial pattern within Hungary, so that a series of maps are being produced, more or less by decade, but not spanning the important divide introduced by the fall of Communism in the Eastern bloc. The spatial patterns were modelled reasonably accurately, and so we are able to identify crucial predictor variables. We are examining time series of AVHRR data for any signs that these indicator variables have changed over time. Whether or not they have, we will be contributing to the E£DEN TBD's important efforts to tease out the relative roles of environmental and other (e.g. political, economic, social) changes in determining the changing pattern of TBE over time.

ZOOX (HIT)-LEI (NHM and partners). Extension of existing mapping to other areas.

Achieved results: risk maps for two important Leishmaniasis vectors in Europe (*P. perniciosus* and *P. ariasi*) were completed, based on point located data from France and the temporal Fourier processed MODIS data. The risk maps identify most of the Southern regions of France as suitable for both vectors, and parts of

Central/Northern France suitable only for *P. perniciosus*. The key predictor variable for each species is the maximum of the night-time Land Surface Temperature, indicating that night-time conditions need to be sufficiently warm for these two vectors to survive. An artefactual consequence of this is that major cities (Paris/London) appear to be at a high risk of leishmaniasis; this is solely because their night-time temperatures are higher than in the surrounding areas (the 'heat-island' effect).

ZOOX (HIT)- UCL – LEI and partners. Integration of modelling approaches applied to Leishmaniasis.

Achieved results: this is our first integrated analysis effort where the HITs collaborate with each other and with an SP with a good data set. For various reasons the final provision of the dataset was delayed, and with it this integrated activity. The dataset has now been provided and some preliminary results may be available before the AGM in Morocco.

In summary, whilst progress has not been as good as we would have liked this calendar year, it has been good considering the difficulty of recruiting suitable post-doc skills for the LRRS team.

Deliverables

D HRRS01 – High resolution image analysis for each selected site (M36). Analyses of sites selected so far are completed (ROBO-Belgium, MAL, LEI, TBD) or are ongoing (ROBO-Finland).

D HRRS02 – Landscape analysis and environmental change analysis for selected HR sites (M36). This is ongoing. Sites selected so far have been analysed (ROBO-hantavirus, MAL, TBD, ROBO-cowpox) or will be in the near future (ROBO-Finland). Environmental change has been analysed for LEI.

D HRRS03 – Conceptual model for each disease under study in EDEN (status reported in next annual report) (M36). Conceptual models that link the different agents responsible for disease transmission (vectors, hosts, humans, environment) have been elaborated in collaboration with the vertical sub-projects for ROBO, MAL and TBD.

D HRRS04 – On the job HRRS technology transfer (M36). Completed via collaborations with vertical subprojects.

D LRRS01 – Fourier processed LRRS MODIS data archive established (M27) and regularly updated (+12m). Updating on a continuous basis is now well under control. We have an arrangement with NASA to push the data to us, rather than us pulling them. Currently MODIS image processing by NASA is virtually up-to-date, and the backlog has been cleared.

D LRRS02 – Pan-European maps of eco-climatic seasonality signals (M36). Completed and available.

D LRRS03 – Pan-European maps of space-time environmental changes (M36). Completed and available as filtered images where only significant linear trends (and their values) are saved. This enables us to identify areas of change, the direction and amount of those changes.

D LRRS04 – Information-theoretic models for several EDEN diseases (M30). Completed with EDEN ROBO, LEI and TBD teams.

D LRRS05 – On the job technology transfer (M36). We received visits from the Modelling HIT and the EDEN ROBO team.

D LRRS06 – First process-based vector models for candidate diseases (M30). This work has been delayed due to a shortage of seasonal data for EDEN's vectors.

D LRRS07 – Data-fusion techniques for selected imagery developed (M36). Techniques have been developed but not yet applied.

D LRRS08 – Analysis of changes detected through moving windowed Fourier techniques (M36). This underpins LRRS03, above. The analysed images show areas of significant environmental change across Europe in the period 1982-1999 from TFA-processed AVHRR imagery. The results appear to make a great deal of sense; for example they show that the phase (timing) of the peak of the annual cycles of both Land Surface Temperature (LST) and the Normalised Difference Vegetation Index (NDVI) have advanced in this period of time (i.e. 'earlier Springs').

Milestones and expected result

M RS02 – MODIS data are Fourier processed and available to be included in spatial models (M27). MODISv4 data processed and available. MODIS v.5 data being processed (entire time series was available only from

October 2008)
M RS03 – Strategy document enables to develop integrated approaches of risk assessment linking high resolution information with low resolution surrogates of risk (M27). The final format of the strategy document will be the proceedings of the EDEN International Conference to be held in May 2010 at the end of the requested 7 month no-cost extension.
M RS04 – Conceptual models for selected diseases enable to relate epidemiological variables with HRRS landscape analysis (M39). Conceptual models that link the different agents responsible for disease transmission (vectors, hosts, humans and the environment) have been elaborated in collaboration with the vertical sub-projects for ROBO, MAL and TBD. The conceptual models are being confronted to results of empirical studies. For ROBO-hantavirus statistical models were consistent with the conceptual model integrating the direct and indirect transmission process. For Malaria, a multi-agent simulation that integrates the different agents, their behaviours and relationships presented in the conceptual model has been developed.

WP 6.3 – Horizontal PhD Program

Overview MatMod

1. Work done with vertical teams:

- Malaria team: Started to collaborate to produce R0-risk maps for malaria in two different areas: southern France (malaria absent), and an area in Turkey (malaria present), to compare performance of risk-mapping methodology (as developed in previous reporting period). This will commence in the next working period.

- Leishmania team: Work on merging the methodology of R0-risk maps (see 2 below) with techniques from the high resolution remote sensing horizontal team are devoted to produce R0-risk maps for two areas of France for leishmania. The data problems mentioned in the previous report have been solved and all available data have been received in October 2008 allowing final progress to be made on this important EDEN integration of all methodological horizontal teams in an effort to obtain insight into a fundamental question of a vertical team. This should then play the role of a 'blueprint' for other vertical teams. Work will commence in November 2008. First results will be presented at the next AGM in Marrakesh. The results should lead to a joint manuscript in early 2009.

2. Work done on the methodology of risk maps:

A manuscript containing the methodology (integration of field data on vector abundance, low-resolution modelling, and R_0-modelling), based on bluetongue virus as a case study to guide development, was prepared and submitted to Proc. R. Soc. B. The manuscript was rejected after review (on data quality) and has been revised extensively and was submitted to Epidemics in October 2008.

Publications of EDEN-MatMod (with an EDEN number)

HARTEMINK N.A., PURSE B.V., MEISWINKEL R., BROWN H.E., DE KOEIJER A., ELBERS A.R.W., BOENDER G.J., ROGERS D.J., JHEESTERBEEK J.A.P., (submitted). Mapping the basic reproduction number (R_0) for vector-borne diseases; a case study on bluetongue virus

HARTEMINK, N.A., RANDOLPH, DAVIS S., HEESTERBEEK S.A. & J.A.P. (2008). The basic reproduction number for complex natural systems: defining R0 for tick-borne infections. *American Naturalist*, 171, 743-754.

HARTEMINK, N.A., DAVIS, S.A., REITER, P., HUBALEK, Z. & J.A.P. HEESTERBEEK (2007). The importance of bird-bird transmission for the establishment of West Nile virus. *Vector-borne and Zoonotic Diseases*, 7, 5765-584.

Meetings where papers involving EDEN-MatMod ideas have been presented

In this reporting period: EDEN annual meeting (Brno), EMOP conference (Paris), Conference on R_0 (Paris), workshop on zoonoses (Liverpool), Workshop on veterinary epidemiology (Wageningen), Veterinary Science Day (Utrecht)

Overview BIODIV

During the last 12 months term, HIT BioDiv activities have concentrated their efforts on the writing of scientific publications based on results acquired from previous years. In particular, HIT BioDiv Benjamin Roche' PhD student passed with honorific distinction last June 2008, 27th his oral defence at Montpellier University, and he got a short term employment contract at FAO in Roma to work on avian flu dynamics (FAO proposed him a long-term employment contract he refused), and he is now post-doctoral fellow at Athens University (starting date: 08/01/08). Overall nine papers have been written during this term, 3 of them are book's chapters (none are EDEN-referenced) and 6 are scientific publications (3 are actually EDEN-referenced and 1 will be soon).
One paper (papers' ranking below follows the present description) has more specifically studied the impacts of biological diversity and its species composition upon the circulation of some pathogenic agents. Here, in both using theory and data (West Nile Virus, LP avian flu virus, Lyme disease), we have shown what can be the impacts of community species patterns and its heterogeneity on disease transmission for both zoonotic and vector-borne diseases. Lyme disease, low pathogenic avian flu viruses and West Nile fever in real conditions have been compared with models issued from our study.
A second work has focused on the impacts of host bird species reservoirs composition, diversity and dynamics on low pathogenic (LP) avian flu viruses in Southern France. In this investigation, 3 scenarios of LP avian flu viruses transmission have been tested, ie. by migratory birds, by the aquatic environment and by direct contacts between bird individuals. Results suggest, in both using mathematical modelling and an impressive data set for both bird species and avian flu virus types, that the transmission patterns driving the dynamics of infection in the bird sanctuary Camargue delta involve both a density-dependent and a water-borne transmission. However, water-borne transmission appears to be the main determinant of the disease dynamics and observed prevalence level, thus rejecting the hypothesis of a transmission of these kinds of virus through transcontinental bird migrations from Africa to Europe.
A third work has focused on the effects of space and its heterogeneity on disease transmission in proposing a new type of computational modelling, and we developed a multi-agent based system model for vector-borne disease transmission in a realistic spatial environment. This work is available as a free-software on the net. Of the same vein, a fourth paper has more deeply analyzed the spatio-temporal dynamics of vector-borne diseases in heterogeneous patchy landscapes. In this study, we focused on one particular aspect of disease behaviour in fragmented landscapes, which is disease (un)predictability to emergence and spread. The question of the advisability of opting for a certain type of disease control policy when facing unpredictable disease behaviour has then been discussed.
A fifth paper has studied the influence of trophic chains and ecological interactions between species and individuals on disease transmission from both a pure theoretical side and a more empirical one. The empirical study is an extra from EDEN, and this is the main reason why we did not ask for an EDEN number for this paper. Since the philosophy of this work is EDEN-based, we then present it. Using mathematical modelling supported by quantitative data from 27 different localities in Western Africa, we have developed an optimal ecological web model which represents how Mycobacterium ulcerans agent might be transmitted within local aquatic host communities from host individual to host individual. Besides its applied aspect for the understanding of *M. ulcerans* ecology, this study underlines the efficiency of parasite transmission through ecological webs, a notion which could be highly relevant also to many other kinds of infectious diseases.
A sixth paper, to be finished soon, has used a comparative phylogenetic analysis to show the existence of a negative relationship between average body mass of bird species and their seroprevalence for West Nile virus in captive avian species. These findings suggest the existence of an allometric relationship between body mass and intrinsic susceptibility across bird species that it could be explained by the increase of immune system complexity with body mass in bird species. This work will facilitate the understanding of West Nile virus transmission in wildlife, and for which the body mass of bird species seems to be a good indicator of disease seroprevalence. This work is still in progress through a positive collaboration with partners from Cirad and EID.

Three books' chapters (2 in French and 1 in English) have been written, all of them addressing the impacts of global environmental change and their interactions (habitat fragmentation, land use changes, bioinvasions,…) on host-pathogen associations, with major consequences in health ecology (emergence and re-emergence, evolution of virulence and transmission,…). In this series of publications, we demonstrate how biodiversity sciences, epidemiological theory and evolutionary ecology need to cross-fertilize together to better understand disease emergence and transmission, notably when the environment is facing some important perturbations and alterations.

Publications of EDEN-BIODIV (with an EDEN number)

- Community ecology and epidemiology of infectious diseases in wildlife: a theoretical approach. Roche, B., Dobson, A.P., and Guégan, J.F. (submitted). This publication is catalogued by the EDEN Steering Committee as EDEN0089.
- Avian influenza dynamics in wild birds are driven by water-borne transmission. Roche, B., Lebarbenchon, C., Gauthier-Clerc, M., Chang, C., Thomas, F., Renaud, F., van der Werf, S., and Guégan J.F. (2009). Infection, Genetics and Epidemiology (in press). This publication is catalogued by the EDEN Steering Committee as EDEN0048.
- Multi-agent based systems in epidemiology: a first step for computational biology in the study of vector-borne disease. Roche, B., Guégan, J.F., and Bousquet, F. BMC BioInformatics 9: 435. Online. This publication is catalogued by the EDEN Steering Committee as EDEN0094.
- On the predictability of vector-borne diseases in wildlife. Roche, B., Real. L.A., Bousquet, F., and Guégan, J.F. (pre-ultimate draft – an EDEN reference number will be asked).
- Pathogen transmission through local food webs: the case study of Mycobacterium ulcerans. Roche, B., Benbow, M.E., Merritt, R., Kimbirauskas, R., McIntosh, M., Small, P.L.C., Williamson, H., and Guégan, J.F. PLoS Neglected Tropical Diseases (in review).
- Disentangling the effects of intrinsic susceptibility and ecological factors for epidemic patterns. The case of West Nile Fever. Roche, B., Elguero, E., Balança, G., Morand, S., Guégan, J.F., and Gaidet, N. (To be submitted soon– an EDEN reference number will be asked).
- How the biodiversity sciences may aid biological tools and ecological engineering to assess the impact of climatic change. Morand, S., and Guégan, J.F. (2008). Revue scientifique et technique de l'Office international des Epizooties 27: 355-366.
- Ecologie et épidémiologie : un mariage de raison(s) pour une histoire d'échelle(s). Guégan, J.F. et Morand, S. (2008). In Introduction à l'épidémiologie intégrative des maladies infectieuses et parasitaires. (eds. Guégan, J.F., et Choisy, M.). De Boeck Université, Louvain, Belgique (in press).
- Comprendre la complexité des systèmes épidémiologiques naturels. Une introduction à l'épidémiologie des communautés. Roche, B., et Guégan, J.F. (2008). In Introduction à l'épidémiologie intégrative des maladies infectieuses et parasitaires (eds. Guégan, J.F., et Choisy, M.). De Boeck Université, Louvain, Belgique (in press).

Meetings where papers involving EDEN-BIODIV ideas have been presented

- Guégan J.F. and Roche B. Impacts of community species structure on disease dynamics in wildlife. BestNET-DIVERSITAS-EcoHealth-AgTrans Workshop, May 28th-30th 2008, Global Institute of Sustainability, Arizona State University, Tempe, USA. (Oral communication). Invited.
- Guégan J.F. Presentation of the EDEN project at both the French Ministry of Health and the French Ministry of Agriculture (for the later on behalf of R. Lancelot), Paris, France, 2008.
- Roche B. and Guégan J.F. Avian influenza is water-borne transmitted. Workshop ``Shared traits, extended phenotypes or GxGxE interactions'', 5 December 2007, Paris, France (Oral communication).
- Roche B. and Guégan J.F. Ecosystem dynamics may drive disease dynamics in wildlife. Seminar at NSF/NIH Fogarty Center, 27 August 2007, Washington D.C., USA (Oral communication). Invited.
- Roche B. and Guégan J.F. Community structure and composition: Implications for wildlife disease dynamics. Ecological Society of America Annual meeting, 5-10 August 2007, San Jose, USA. (Oral communication).
- Roche B. and Guégan J.F. Spatial behavior of multi-host vector-borne diseases. Ecology and Evolution of Infectious Diseases Conference, Penn State, USA, 2006. (Oral communication).

- Roche B. and Guégan J.F. Impact of community composition and structure on disease dynamics in wildlife. EcoHealth One Conference. Madison, Wisconsin, USA, 2006. (Oral communication).

Work package number	WP6.3	Start date or starting event:		37			
Participant id	UCL	FVM	IRD				Total
Person-months per participant	18	18	18				54

Objectives

The overall objective is to train EU students to PhD level in a variety of advanced, multi-disciplinary analytical techniques and to spread knowledge of these techniques throughout the EDEN community. Topics include epidemiological modeling, environmental change modeling and community ecology of infectious diseases.

The specific objectives of the next 18th months periods are:

- For the 'Risk maps and Disease Modelling' PhD: to construct generic epidemiological models for vector transmitted disease agents and geographic maps for such systems;

- For the 'Environmental changes at the landscape scale' PhD: to analyse the effects of land use change on the distributions of disease vectors and vector-borne diseases;

- For the ' biodiversity and health ' PhD: to evaluate the impact of the biodiversity (in term of local and regional vector/reservoir species richness and composition) on locally infectious disease transmission.

Work performed in the previous reporting periods

Students were selected for HIT PhD's on the following research topics: Risk maps and vector modelling; Environmental change at the landscape scale; the linkages between biodiversity and health. The students joined the different workshops (Data management, GIS and Remote Sensing Workshop, Mathematical Modelling workshops, PHD Meeting) and contributed to the discussions on a complementary approach. In addition, meetings between HIT PhD and vertical SP teams have been organized. Maintaining this network will be an important objective of next reporting period.

MatMod: Two scientific papers are in press in respectable scientific journals: one is collaboration with the West Nile Virus team, the other with the TBD team. A third manuscript, where the spatial methodology for risk mapping is developed, is collaboration with the Low Resolution modeling team. Meetings have been held with the Leishmania team and the High resolution team. This will lead to collaboration on risk maps for Leishmania in the coming period.

BioDiv: A first paper has been published and an additional series of papers are in preparation using results obtained to date. Collaborations are ongoing with the EDEN WNV and HRRS teams and scientific exchanges were organized with other PhD students: N. Ponçon (SP MAL), G. L'Ambert (SP WN) and K. Tersago (SP Robo). In addition data on West Nile virus, avian flu and Lyme disease originated outside EDEN were used to develop modeling approaches and test hypotheses.

Description of work

MatMod: Planned activities next period for the R0 mathematical modelling are: finish manuscript developing the spatial risk methodology using Bluetongue Virus as the example. Develop risk maps based on this methodology and combining with High and Low resolution modelling teams for the Leishmania team. It is envisaged that the PhD thesis will be completed in the early summer of 2009, within the standard four-year period that is required in the Netherlands to reach a sufficiently high level of quality and quantity of research output necessary for a PhD.

Achieved results: In previous accounting periods we collaborated with WNV and TBD vertical teams (both leading to scientific papers). In the third period we collaborated with the Leishmania vertical team (will be concluded in the final accounting period with a joint publication) and we started collaboration with the malaria vertical team. The work with the Leishmania team for the first time combines the expertise of a vertical team with that of three Hits (Mod, LR and HR). Methodology was developed using BTV as an example (due to problems with Leishmania data that have now been solved).

BioDiv: HIT BioDiv: With G. L'Ambert, in charge of contributing to the development of the vector species-habitat characteristics data-base for the Camargue area, development of the ABM-GIS interactive system for West Nile Fever transmission in wildlife.

Achieved results: This part of the work is still in progress due to some administrative problems with G. L'Ambert's (from EID) registration at Montpellier University. J.F. Guégan is now scientific advisor (he acts as a deputy for the School of Doctoral Studies) for L'Ambert's thesis committee. Vector-species habitat characteristics for the Camargue area will be added to the ABM-GIS interactive system for West Nile Fever transmission in wildlife within the next two/three months with the help of Annelise Tran from HRRS team. Let us note here that the ABM has been already published, and it is available at http://roche.ben.googlepages.com (programming language: Java on a Swarm platform).

With K. Tersago from SP RoBo, a collaboration to model the effect of local reservoir species community patterns on some RoBo disease transmission, i.e. Puumula virus, other types of Hantavirus, in wildlife will be developed.

Achieved results: This collaboration has failed to be achieved due to the fact that K. Tersago decided to pursue her research work on the impacts of local reservoir species community patterns on some RoBo disease transmission, ie. the dilution effect theory, alone.

Deliverables

D 45 – Respective methodologies included in strategy document (M30)

HIT BioDiv An Agent-based model has been published in the international journal *BMC BioInformatics*, and it is freely available at http://roche.ben.googlepages.com (programming language: Java on a Swarm platform). Reference paper is: Multi-agent based systems in epidemiology: a first step for computational biology in the study of vector-borne disease. BMC BioInformatics 9: 435. Work coupling this ABM to a GIS for west Nile virus transmission in the Camargue area is still in progress, and should be achieved within the next months.

HIT MatMod: Achieved and ongoing

D 46 – Respective PhD progress reports (M36)

HIT BioDiv Benjamin Roche' PhD student passed with honorific distinction last June 2008, 27th his oral defence at Montpellier University. PhD thesis report is entitled "Complexité des écosystèmes, dynamique de la diversité biologique et maladies infectieuses", which is exactly the translation of "Ecosystem dynamics, biodiversity and infectious disease" the main scope for HIT BioDiv (a summary of PhD report is in preparation for the EDEN website, and it will be available shortly). The two examiners for the PhD oral defence were Pr. Andy Dobson from Princeton University, a top-leader scientist in this field of research, and Dr. Christian Lannou from the French institute for agriculture sciences, because this type of research can also be of some strong interests in plant disease transmission within crop fields. The EDEN consortium was represented by former project coordinator, Dr. Stéphane de la Rocque from both Cirad and FAO. Benjamin Roche then got a short term employment contract at FAO in Roma to work on avian flu population dynamics, and FAO authorities proposed him for a long-term employment contrat he refused. B. Roche is now post-postoral fellow at Athens University, GE, USA (starting date: 08/01/08) in both the Odum School of Ecology and the Centre for Tropical and Emerging Global Diseases, and he is funded with a National Science Foundation postdoc grant. B. Roche will pursue his research work on the linkages between ecosystem dynamics, biodiversity and infectious disease, and thus he will contribute to the dissemination of EDEN epistemology and work at international scale.

HIT MatMod: Achieved and ongoing

Milestones and expected result

M34 - The discussions at the annual meeting and PhD meeting strengthen the PhD network and contribute to the wider dissemination of the EDEN approach (M45)

HITBioDiv team strongly contributed to the dissemination of their ideas on the linkages between ecosystem dynamics, biodiversity and infectious diseases within the EDEN network. More specically, JF Guégan was invited to give an invited Conference at Tempe, Arizona State University, within a BestNet (National Science Foundation)-DIVERSITAS-EcoHealth-AgTrans workshop (PI: Charles Perrings, ASU) on Analyzing the Role of Agricultural transformation and Invasive Species in disease Emergence, during which he exposed the main findings from EDEN HIT BioDiv research initiatives. A report to the American NSF is in preparation by now, and in which the EDEN HIT BioDiv results will be presented and discussed.

WP 7 – Overall integration: tools and scenarios

Deliverable review

The EDEN strategy document originally aimed at documenting the improvement of state of the art achieved by EDEN. As seen in this report, ca. 130 papers are currently in the peer review system from which 79 published pdf's are available, 12 are in press and 32 are submitted. Highlights from these and ongoing work will be presented at the EDEN AGM (Marrakesh, January, 2009) during the PhD meeting (20+ oral presentations), during the plenary AGM (30+ oral presentations) and during the poster sessions (30+ posters). At the plenary part of the AGM, each of the SP leaders will introduce the work achieved by summarizing major scientific results and putting them into the general perspectives of the SP objectives and the more general EDEN objectives. In the final year of the project, most efforts will focus further on publishing EDEN results and all sub-projects will devote a significant part of their SP meetings at the AGM to discuss their last year publication plan with focus on integrative papers joining data from different teams within and between sub-projects. This will be the onset to the final international EDEN conference to be held in Montpellier in May 2010 at the end of the requested 7 month no-cost extension. It therefore is suggested to:

1. Include the International EDEN conference as an EDEN deliverable.
2. To publish the final version of the EDEN strategy document as an e-book including the fully documented proceedings of that conference.

Work package number	WP7	Start date or starting event:			37		
Participant id	ZOOX	FVM	UCL	CIRAD	IRD	IPP	METLA
Person-months per participant	2	2	2	2	2	1	1
Participant id	NHM	AFR	Euro-AEGIS	Avia-GIS			Total
Person-months per participant	1	1	1	1			15

Objectives

The overall objective of this work package is to integrate the information from the Sub-Projects on individual diseases through the activities of the Horizontal integration teams in order to be able to achieve the general objectives of EDEN which are (a) to identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change, which can influence the spatial and temporal distribution and dynamics of pathogenic agents in general and (b) through the development of generic tools to provide support for monitoring systems, predictive emergence and spread models and global and regional preventive early warning systems.

This is achieved through the writing of a RTD strategy document (SD). During the first year a SD draft was written by the central management team based on discussions prior to the start of EDEN, at the Kick-Off meeting, during EDEN integration workshops and at the Steering Committee meetings. In this reporting period the second version will be finalised. The dissemination of outputs will be organized under WP8.

Work performed in the previous reporting periods

Two versions of the strategy document have been produced and discussed. The current status of the different chapters is summarized in the table below.

Chapter 1 – EDEN project	Completed
Chapter 2 – State of the Art per Sub-project and Horizontal Integration Team	Completed
Chapter 3 – EDEN strategy in theory	Completed
Chapters 4-9 – Individual disease chapters	In progress
Chapter 10 – Generic models	Not started
Chapter 11 – Information systems, tools and scenarios	Notes included
Chapter 12 – EDEN dissemination plan	Completed
Appendix A – EDEN strategy in a nutshell	Completed
Appendix B – Elements of a dissemination plan	Completed

Description of work

In the next reporting period the series of identified key-problems will be further addressed in liaison with ECDC, DG-SANCO and GEOSS.

The first problem is the lack of estimates of key parameters of disease transmission; these can only be established through carefully designed experiments, carried out either by EDEN teams or by partners in other (non-EDEN) institutes. The second problem is the best type of modelling approach to use. This will be problem specific; SIR models (Susceptible / Infected / Recovered) for the spread of diseases, and their derivatives may be satisfactory for disease transmission, whilst agent-based models will be required for understanding land-use and land-cover changes over time. Finally there is the common problem of obtaining sufficient, accurate Public Health data with which to build and test the models; this can only be addressed by establishing close links with in-country PH services, who might reasonably expect some help or advice in return.

The fact that at least some of these three strands of activities rely on partnerships outside EDEN means that EDEN itself cannot create a stand-alone, functioning DMEWS. Instead EDEN is developing the tools and can recommend the approaches that might be used in constructing such a DMEWS and it is the job of the Strategy Document to illustrate how such a system might be put together, and what might the pitfalls be. An analysis of the results achieved by each sub-project will be conducted and integrated in the disease specific chapters 5-10 to prepare for the discussion on generic models in the final year of the project.

During this reporting period all efforts in this field focused on the publication of peer reviewed papers. As compared to the previous report 48 additional papers have been given an EDEN number, an increase of 64% as compared to the previous annual report (75 papers). Many of these include integrative topics.

Each SP leader will summarise the main findings of her/ his SP at the AGM. This will be done in the general perspective of both the SP specific and EDEN's general objectives. This presentation will be the onset for the detailed review key-note paper which will be presented at the start of each individual session during the EDEN international conference.

Deliverables

D OI03. Strategy document v3.0 – includes chapters on disease specific models provided by EDEN SP leaders (ongoing).

Each SP leader will summarize advances of the state of the art at the Marrakesh AGM.

D OI04. Inputs from SC, AG members, selected international users and the EDEN PhD network members are included in the EDEN strategy document V3.0 (ongoing).

To avoid intellectual property issues, priority was given to publishing peer reviewed papers. The contributions made at the final EDEN conference will be published jointly as the final version of the EDEN strategy document.

Milestones and expected result

M OI04. Strategy document v3.0 is available for comments by SC, AG members, selected international users and the EDEN PhD network (ongoing).

State of the art will be presented for discussion by SC and AG members as well as all EDEN partners during the various sessions at the EDEN annual PhD meeting and AGM.

WP 8 – Management, training & dissemination co-ordination

Deliverable review

No deliverables to be rescheduled.

Work package number	WP8	Start date or starting event:			37		
Participant id	CIRAD	ZOOX	FVM	UCL	Euro-Aegis	IRD	IPP
Person-months per participant	28.5	4	1	1	1	6	1
Participant id	METLA	NHM	ISRA	Avia-GIS			Total
Person-months per participant	1	1	1	7.5			53

Objectives

Because EDEN is a complex project, the consortium has opted for a strong central management and compact Steering Committee. The day to day coordination of EDEN is conducted by three persons: the project coordinator focal point to the EU, the chairman of the Steering Committee (in charge of the scientific coordination) and the secretary of the Steering Committee (in charge of the operational coordination). They are assisted by a management team composed of (i) a financial manager, (ii) an administrative assistant, and (iii) timely inputs from specialists (web-master, project engineer, communication specialist, graphic designer etc.). The overall objective of the central management committee is to co-ordinate the management, training and dissemination activities of EDEN.

Work performed in the previous reporting periods

- Implementation of the EDEN website and SP websites
- EDEN leaflet and poster for dissemination
- Kick-off meeting (in Montpellier): 110 participants
- General annual meetings in Finland, Turkey, and Czechland with on average 120 participants each.
- Three steering-committee meetings per year from 2005 to 2007, two in 2008.
- West Nile meeting in Montpellier with 36 participants.
- R0 workshop in Paris (Oct. 2008), with 125 participants.
- PhD meetings in Paris, Antalya and Brno with 30-50 participants each. Organization of a annual PhD award with a price of 2,000 Euros to attend an international conference.
- Many interviews on radio, TV and articles about EDEN in many media.
- Presentation of EDEN in many international conferences by the coordinator, the Secretary of the SC and the chairman of the SC.

Description of work

Permanent co-ordination tasks include: (a) to act as a permanent contact point with the EU, (b) day-to-day co-ordination & troubleshooting, (c) to provide administrative guidance for partners, (d) to follow up the EDEN milestones, (e) to conduct financial monitoring, (f) to facilitate communication between project teams and (g) to liaise, through EDEN focal persons, for gender, personnel and ethical issues.

The permanent coordination tasks are implemented on a daily basis by the EDEN management team. Scientific issues of general interest are discussed by the coordinator, the chairman of the SC and the secretary of the SC. If needed, proposed solutions are further discussed with SC members through e-mails or eventually at SC meetings. At the occasion of a training course given in Dakar (February 2008), the coordinator met the director of IPD (partner 48) and the representative of IRD (partner 13), and had a meeting with the African platform to find solutions to the data base / data-sharing issues.

Regarding the regular course of the project, the EDEN financial officer and administrative assistant are stand by on a permanent basis to respond to relevant questions and contribute to problem solving. Financial monitoring is achieved through the network of financial officers of partner organizations. A monthly management meeting is held at CIRAD headquarters in Montpellier, France. Key EDEN milestones are followed up at SC level; per SP or HIT each respective leader (all members of SC) is responsible for milestones and deliverables. During this reporting neither gender nor ethical issues were raised by EDEN partners. A careful watch is being kept over progress.

Specific activities linked to meetings and reporting include: (a) to organize and report three annual steering committee meetings (one at AGM, one in June and one in October 2008), (b) to organize the fourth EDEN AGM during the second week of January 2009, the meeting will include a first day plenary session with EDEN scientific presentations open to the international scientific public (advertised through EDEN website and mail list), (c) to organize an EDEN workshop on August 27, 2008, at the Xth European Multicolloquium of Parasitology (EMOP) in Paris, France, (d) to organize annual project reviews and audits.

Two steering-committee meetings (SCM) were organised respectively in Brno (January 2008) and Paris (September 2008). We tried this new frequency following various suggestions, including from EDEN reviewers, and attempts to remedy to the overloaded diaries of all the SCM participants. The drawback was an overlaoded agenda of the Paris SCM. For the last year of the project, we propose to re-establish the frequency of 3 SCM a year. This will mainly be needed to prepare thoroughly the international conference to be held at the end of the project (Montpellier, May 2010).

Just before the Paris SCM, a joint meeting was organised with the Epizone project (FP6): Network of Excellence for Epizootic Disease Diagnosis and Control. The mutual activities were presented and proposals wer made to strengthen the collaboration between the two projects. Epizone was mainly interested by our modelling and integrative approaches. Calls for proposals may be lauched by EPIZONE for small collaborative projects between EDEN and EPIZONE teams.

The coordination was involved in many international conferences and workshops:

- European Society for Vector Ecology (Cambridge, UK, 25-26 March 2008): presentation of EDEN activities and results. On this occasion, the "regular" EDEN presentation was deeply revised to include research results.
- *International Conference on Livestock and Global Climate Change*, Hammamet (Tunisia), 17-19th May 2008: at this conference, the EDEN project was presented with a special focus on emerging, vector-borne diseases affecting livestock. Emphasis was put on two items:
    - Livestock is essential to livelihood in many developing countries: preserving and improving livestock health is an important issue, and vector-borne diseases need more attention.
    - Many human diseases are actually anthropo-zoonoses: they are common to livestock and humans, and in many cases, livestock are reservoir hosts for dangerous human pathogens (e.g., Rift Valley fever).

Climate change will likely deeply alter the epidemiological patterns of many vector-borne diseases in Africa, with possible threats for livestock productivity and human health. We urgently need more resources to have a global understanding of the possible changes, to better alleviate them.

- *INRA-CIRAD International Colloquium*, Paris (OIE), 3rd June 2008: presentation of EDEN integrative approach in front of an audience of directors of national and international agronomic research organisms and universities from > 50 countries worldwide. In this conference attended by the European Commissionner for Science and Research, Janez Potocnik. The lobbying work was pursued to promote EDEN methods and results, and explain the need of EDEN-like projects in developing countries.

- *Euro-Mediterranean scientific network meeting*, Paris, 25th June 2008: presentation of the EDEN integrative approach in front of an audience of directors of national and international research organisms and universities from the Mediterranean region. The lobbying work was pursued to promote EDEN methods and results, and explain the need of EDEN-like projects in the Mediterranean region.

- Organisation of an EDEN satellite meeting (2 * 3 hours) during the Xth European Multicolloquium or Parasitology (Paris, 27 Aug. 2008): participation of the coordinator, the secretary and chairman of the steering committee, as well as several EDEN scientists from France, England, Belgium and the Netherlands. A joint presentation was made with Evelyn Depoortere, ECDC, to show the link between research and expertise, and between research and public-health institutions.

- R_0 workshop in Paris (EDEN, INRA, EHESP, 28-30th October 2008): see above. This workshop was an opportunity to link EDEN with other geographic areas (Africa, Americas, Asia) and to confront the methodological approaches adopted in EDEN and other teams. With this respect, very interesting methodological presentations were made by US teams (e.g., human flu modelling at CDC Atlanta).

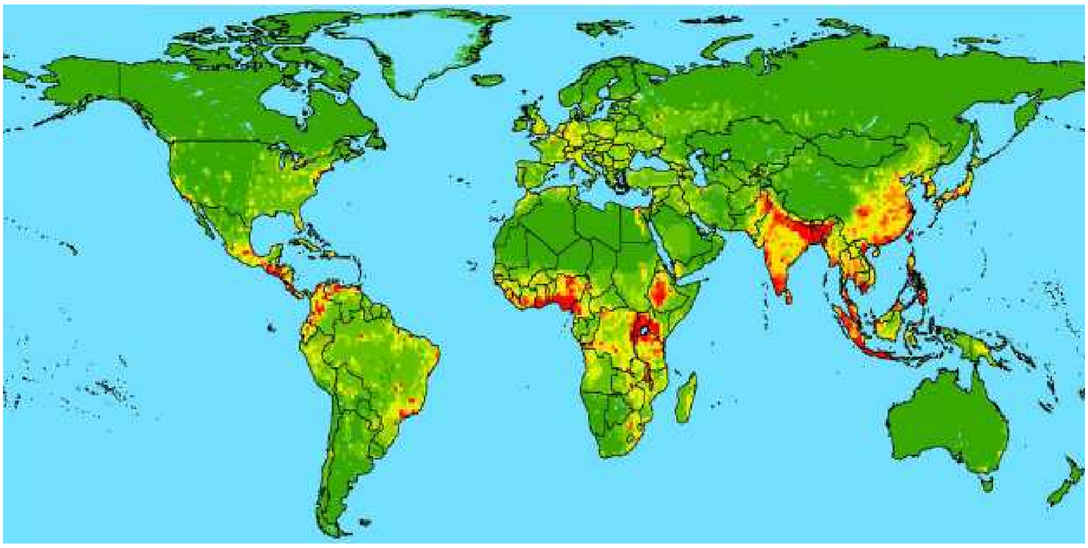


Fig. Coord-O2. Global distribution of relative risk of vector-borne diseases. The relative risk is calculated from regression coefficients and socio-economic, environmental and ecological variables and mapped on a linear scale from green (lower values) to red (higher values). Reproduced from: Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. & Daszak, P. 2008. Global trends in emerging infectious diseases. *Nature*, 451: 990-993, doi:10.1038/nature06536

- Co-organisation with CIRDES (Centre International de Recherche-Développement sur l'élevage en zone sub-humide), CIRAD, IRD and Abomey University (Benin) of an international conference on "Demographic and climatic changes: impact on vector-borne diseases in West Africa". It was held in Ouidah (Benin) at the School of Public Health (24-26th Nov. 2008) and attended by ca. 150 scientists from northern, western and central Africa, and Europe (France, Belgium, The Netherlands, Germany), as well as the students of the *International Entomology Master* co-organised by IRD and Abomey university. The EDEN co-ordinator presented a keynote lecture on EDEN methods and results, and the possible implementation of a similar project in Africa. The conclusion of this communication is that densely-populated areas of Africa (Guinea gulf, Victoria lake basin, Ethiopian highlands…) are high-risk ecosystems for the emergence of vector-borne diseases. This idea was nicely summarised in a recent paper published by Jones et al. (2008) in *Nature* (Fig. Coord-02). Therefore, research and surveillance programmes should be implemented to assess, map and monitor the emergence risks according to environmental, social and economic factors. Indeed, many EDEN teams are already involved in somewhat sparse research programmes in Africa (malaria, trypanosomiasis, Lassa fever, plague, Crimean-Congo haemorragic fever, Rift Valley fever…). Nevertherless, there is a lack of global understanding and generic vision: we definetely miss an ambitious research programme on emerging, vector-borne diseases in Africa!

- Joint meeting of the French Veterinary and overseas academies (Paris, World Organisation of Animal Health, 28th Nov. 2008): presentation of EDEN results on the impact of climate change and other environmental factors on the emergence of vector-borne diseases.
- "France Vétérinaire International" meeting in Paris, Ministry of Agriculture, 4th Dec. 2008: presentation of possible implementation of a research programme on vector-borne diseases in Africa.
- Other attended workshops and conferences include:
    - ECDC, Stockholm, Sweden: European Environment and Epidemiology Network (E3).
    - GEOSS, Brussels, Belgium: GEO European Project Workshop.
    - MERIAL, Tunis, Tunisia: 6th Merial symposium on parasitosis & arthopod-borne diseases. Arthropod-borne Diseases: new challenges for Europe and the Mediterranean basin.
    - UN, Poznan, Poland: UN Climate Change Conference, European Union side events programme, Research into Climate Change Imlpacts on Health around the World.
    - WHO, Madrid, Spain: international public health symposium on environment nd health research.

Specific activities linked to training co-ordination include the follow-up of training activities within the Sub-Projects and by the HIT's. The EDEN PhD network will be further implemented and annual meetings will be organized prior to the AGM. During that meeting each PhD participant will be invited to present a presentation on scientific progress made. Three of these will be peer-selected for presentation at the first day plenary session of the AGM. The AGM participants will select by individual vote the best presentation as 'annual EDEN PhD award'. The price is a funded participation to a relevant international conference (max. €1,500). In addition at the AGM the best poster will be selected by the AGM for an award (€500 in cash).

This policy was pursued. Opportunities were given to PhD students to show their results in international meetings such as EMPO X in Paris (August 2008), and to improve their skills in mathematical modelling (R_0 workshop, including the one-day workshop).

The Advisory Group will continue to provide inputs at AGM: chairing scientific session, participation to debates, selection best poster, and participation to SC meeting.

An attempt was made to reinforce the participation of AG members to the Marrakech annual meeting, January 2009.

Specific activities linked to dissemination are described in the dissemination plan (see http://www.eden-fp6project.net/ (main page) and include:

- The day to day implementation of the EDEN Website: The EDEN website is a gateway to inform the general public and various user groups about EDEN, its activities and successes. It hosts the various EDEN publications (EDEN newsletter, technical guidelines, workshop presentations, papers), restricted parts for internal discussions inside the SP and is linked to the spatial databases and information systems developed as part of the activities of WP 6.1. A special pedagogic section ("EDEN for Kids") will be added for public consultation provided adequate professional is affordable within budget limits. In this section, the selected diseases will be described and the research questions addressed in EDEN are presented in a recreational way.

The master EDEN website (http://www.eden-fp6project.net/) was improved (graphical interface) and regularly updated. Through the website, we receive several requests every week from the general public, teachers, journalists… for information, leaflets, or interviews.

The list of publications was updated and disseminated on many occasions: request from the general audience, teachers, scientists, other European projects, and journalists.

- A document gathering all EDEN publications is available on EDEN website at http://www.eden-fp6project.net (the present document dates back to September 2008. It will be updated in January 2009).
- A web interface was implemented on the Data management website: http://ergodd.zoo.ox.ac.uk/eden/ (fig. Coord-03).
- A simple list of EDEN publications can be automatically produced form a bibliographic database by the coordination and sent on request of anyone interested. This list (as of December 18th 2008) is reproduced as an annex to this document.

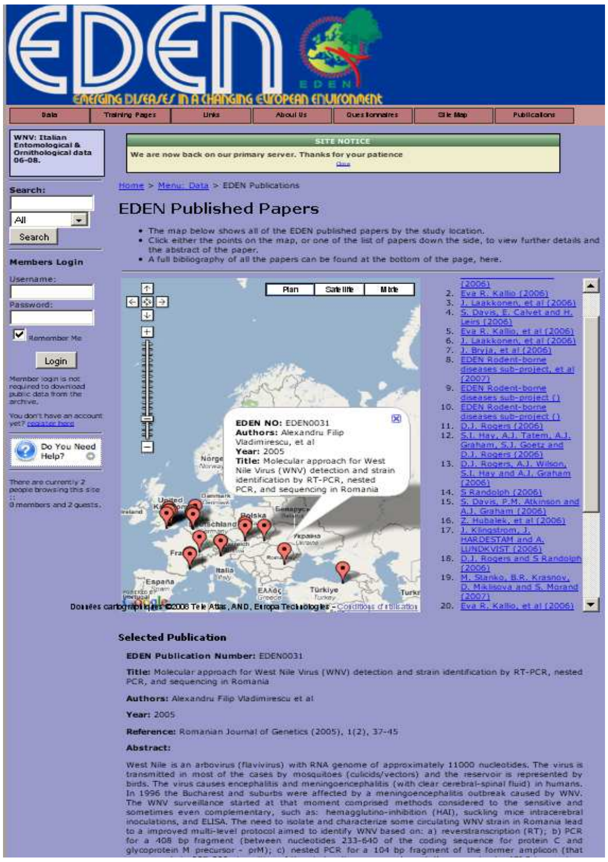


Fig. Coord-03. Web interface to EDEN publications

Edition of the six-monthly newsletters in an electronic format for large dissemination in the "user group" listing and available on the public part of the website. They will be sent to the "potential users" which have been listed. The newsletters include an annotated bibliography (summery plus detailed references) of the accepted EDEN publications. The first newsletter is published after the AGM and the second newsletter after summer.

The edition of the newsletter has been abandoned on profit of improvement of the web sites (general EDEN web site, Data management web site and PhD website), and the maintenance of a comprehensive list of publications (see above).

- The scientific papers published by the EDEN partners on peer reviews will be recorded in a specific database and will be made available on the website. Extended summaries will be disseminated through the newsletters. Once a paper is proposed for publication, a publication number is assigned by the SC secretary and the draft is sent by e-mail for comments by SC members within a week. Past that term the paper is accepted.

Done: see above.

- The participation of EDEN partners at international conferences where the results of EDEN are presented or the project is quoted will be recorded. A selection of potential conferences will also be regularly updated on the Website.

Achieved and documented separately per SP.

It will be the task of the EDEN coordinator to prepare two mandatory reports which will be updated after every subsequent 18 month period: a report on raising public participation and awareness and a report on gender action plan.

These reports will be updated during the last reporting period.

Finally, the Management will place emphasis on improving the collaboration with existing or proposed projects in line with the objectives of EDEN. It will also facilitate the answer of the consortium to the EC requests for the valorisation of its policies and strategy.

Links were made with the coordinators / steering committees of two FP6 projects:

- The integrated project CIRCE (climate change and impact research: the Mediterranean environment)

- The network of excellence EPIZONE (Network of excellence for epizootic disease diagnosis and control)

Both were interested in EDEN's expertise on the methodological aspects of assessing the effect of climate change (or other changes) on human (CIRCE) or animal (EPIZONE) health. It was decided that the most useful follow-up would be a cross-participation in annual meetings and invitation in workshops to present EDEN methods and results. This will take place in 2008, when more mature results are available for EDEN.

Immediately before the Paris SCM, a joint meeting was organised with the Epizone project (FP6): Network of Excellence for Epizootic Disease Diagnosis and Control. The mutual activities were presented and proposals were made to strengthen the collaboration between both projects. Epizone was mainly interested by our modelling and integrative approaches. Calls for proposals may be lauched by EPIZONE for small collaborative projects between EDEN and EPIZONE teams. The EPIZONE coordination team was also invited to the EDEN annual meeting.

The coordinator attended a workshop organised by the CIRCE project (Athens, June 2008) where he presented EDEN methods and results. It was an opportunity to pursue the dialog with Kris Ebi, one of the external reviewers of EDEN in 2008, and Tanja Wolf, from the European office of WHO.

Discussions began with ESA to implement a development program combining EDEN results and expertise together with space technology tools (remote sensing, GPS…). Two meetings were organised in Paris at ESA headquarters with high-level ESA representatives, including C. Haignerée, special advisor for ESA DG and former French ministry of research. A workshop was organised in Luxembourg (October 2007) with ESA, DG-Sanco, EFSA, WHO and several EDEN partners. Following this workshop, it was decided to prepare a pilot project to demonstrate the proof of concept of an early-warning system to be used by national public-health agencies.

These discussions were further pursued in the framework of the ESA IAP, a document was produced using TBE as an example and Sweden and Austria as test cases. A workshop was held at ESA Noordwijk, Netherlands, to discuss the operational details of such a project. As a result the topic was presented as a bankable project at the ESA IAP ministerial meeting and a call for a feasibility study will be made public in January 2010.

Following a workshop held at ECDC (Stockholm) in March 2007, the EDEN coordination successfully replied to an expertise tender released by ECDC on the "Assessment of magnitude and importance of vector-borne diseases in Europe". The consortium involves many EDEN partners. The final report is expected for mid-2008.

The expertise was achieved as expected. The final report was submitted in October 2008. It should be shortly published by ECDC. Two special issues of the journal "Eurosurveillance" are planned as a follow on to this work. They will be devoted to the peer review publication of disease reviews.

Deliverables

D MAN05 – EDEN Newsletter 6 – AGM presentations (M30)

Canceled. Replaced by new versions of EDEN poster and leaflet.

D MAN06 – Mandatory report on raising public participation and awareness (M30)

To be finalized in 2009.

D MAN07 – Mandatory report on gender action (M30)

Initial version is available on EDEN web site. Final version will be prepared in 2009.

D MAN12 – Third Annual PhD network meeting (M39)

Done

D MAN13 – Annual meeting AGM3 in Brno Czech Republic (M39) Done D MAN14 – EDEN SC and AG meeting at AGM3 (M39) Done D MAN15 – EDEN Newsletter 8 – Publications update (M42) Canceled. Replaced by new versions of EDEN poster and leaflet.
Milestones and expected result M MAN08 – Partners provide timely inputs for Newsletters (M27-31-39). Canceled M MAN09 – The updated EDEN Dissemination plan takes into consideration EDEN's successes and failures in that field of activities and enables the implementation of an improved dissemination strategy (M38). Final version expected in 2009.

Summary tables deliverables and milestones

Table 1: Deliverables List

List all deliverables, giving date of submission and any proposed revision to plans.

Del. no.	Deliverable name	Workpackage no.	Date due	Actual/Forecast delivery date	Lead contractor
D TBD 1	Up-date to end 2008 of spatial database of standardized climatic data for each TBD partner country	TBD 1	54	60 In progress	ZOOX
D TBD 2	Land cover/land use change analysis maps for selected countries at the national, sub-national and local scale	TBD 1	36	48 Done	ZOOX
D TBD 3	National datasets of historical (1970-present) tick change patterns	TBD 2	12	12 Done	LMU
D TBD 4	Standardised field sampling protocols	TBD 2	15	15 Done	LMU
D TBD 5	New advanced molecular diagnostic techniques and protocols for tick-borne pathogens and tick blood meal identity	TBD 2	18	30 Done	LMU
D TBD 6	Standardised database, updated to end 2008, of national records of historical trends in local and regional incidence of TBDs	TBD 3	12	60 In progress	PHA
D TBD 7	Standardised database of national records of historical trends in changes in relevant public health activities	TBD 3	12	16 Done	PHA
D TBD 8	Standardised database of national records of historical trends in sociological indices of behaviour relevant to exposure to TBDs	TBD 3	26	42 Done	PHA
D TBD 9	Completed sociological questionnaires, as necessary.	TBD 3	18	abandoned	PHA
D TBD 10	Grids and protocols for trapping rodents at selected sites in Italy and Slovakia.	TBD 4	15	15	CEA
D TBD 11	Maps of changing patterns of local and regional deer abundance.	TBD 4	26	46 Done	CEA/ZOOX/ Euro-AEGIS
D TBD 12	Maps of changing patterns of local and regional livestock abundance.	TBD 4	26	46 Done	CEA/ZOOX/ Euro-AEGIS
D TBD 13	An up-dated common multi-national geo-referenced database of epidemiological, environmental, biological and sociological information, 1970-present.	TBD 5	18	60 In progress	ZOOX
D TBD 14	Preliminary analysis of spatial patterns of changed TBD incidence, 1970-present.	TBD 5	18	24 Done	ZOOX
D TBD 15	Monthly counts of questing ticks at each site within each partner country over months 37-48.	TBD 2	48	60 In progress	IVB
D TBD 16	Final measures of the geographically variable prevalence of infection with tick-borne pathogens in ticks.	TBD 2	30	54 In progress	IVB
D TBD 17	Information on tick blood meal identity in relation to infection prevalence.	TBD 2	30	54 Unlikely	Neuchâtel
D TBD 18	Third annual pattern of rodent and tick population dynamics, and seasonal prevalence of infection.	TBD 4	48	60 In progress	CEA/SAS
D TBD 19	Database with ROBO and HRRS on rodent populations and viral infection prevalence related to habitat changes in Latvia.	TBD 4	36	60 In progress	ZOOX/ ROBO
D TBD 20	Time series and multi-variate regression analyses to establish correlations, and possible causality, between the epidemiological, environmental and sociological data.	TBD 5	36	36-48 Done	ZOOX
D TBD 21	Analysis of relationship between TBE incidence, landscape structure, land use and human habitation at local scales	TBD 1	48	67 In progress	ZOOX/UCL
D TBD22	Indices of deer (and wild boar) abundance at each site where ticks are sampled.	TBD 4	48	52 Unlikely	CEA
D TBD23	Analysis of public health activities in relation to TBE upsurge in the Baltic States (M42).	TBD 3	36	42 Done	ZOOX
D TBD24	Sero-surveys of wildlife as indicators of the presence of zoonotic cycles of TBDs (M42).	TBD 4	48	48 Done	IVB

D TBD25	TBE risk maps for various countries	TBD 5	54	67 In progress	ZOOX
D ROBO 1	Pan-European regional (1-8km raster data and vector data) spatial data base on masting, forest resources and snow cover.	ROBO 1	12	60 In progress	UA
D ROBO 2	Processed high resolution land-use and land-cover satellite imagery for each field site.	ROBO 1	12	60 Mostly done	UA
D ROBO 3	Regional model on masting dynamics.	ROBO 1	18	67 Mostly done	UA
D ROBO 4	Regional model on forest fragmentation patterns.	ROBO 1	18	67 Mostly done	UA
D ROBO 5	Regional model on snow cover trends.	ROBO 1	18	48 Unlikely	UA
D ROBO 6	Ground-supervised local high resolution landscape change models for study sites in Central Finland, Belgium, Central Sweden, Southern France and Northern Italy. Latvia	ROBO 1	18	67 In progress	UA
D ROBO 7	Standardised human diagnostics protocols.	ROBO 3	4	48 Done	UH.HI
D ROBO 8	First generation maps of observed human rodent-borne virus presence in Europe.	ROBO 3	12	48 Done	UH.HI
D ROBO 9	Second generation maps of observed human rodent-borne virus presence and serological prevalence in Europe.	ROBO 3	18	30 Done	UH.HI
D ROBO 10	Standardised diagnostic protocol for virus and rodent molecular analyses.	ROBO 4	4	60 In progress	METLA
D ROBO 11	First generation maps showing state of the art knowledge of rodent and virus distributions, their phylogeography, regional dynamic patterns, and gaps in coverage.	ROBO 4	12	12 Done	METLA
D ROBO 12	First results on the virus persistence outside the rodent hosts.	ROBO 4	18	18 Done	METLA
D ROBO 13	Second generation maps of rodent and virus distributions including cross-sectional sampling results.	ROBO 4	18	67 In progress	METLA
D ROBO 14	GIS analysis of spatial data sets collated at both high resolution longitudinal study sites.	ROBO 4	18	67 In progress	METLA
D ROBO 15	Exploratory area-wide GIS model for the testing of ROBO risk assessment approaches.	ROBO 5	18	67 In progress	UA
D ROBO 16	Exploratory local high resolution GIS model for each field site for the testing of spatial epidemiology hypothesis and health-environment relationships.	ROBO 5	18	67 In progress	UA
D ROBO 17	Basic protocol on the applicability of MHC genetics in rodent-virus interaction	ROBO 4	30	30 Done	METLA
D ROBO 18	Research plan to study the affect of forestation of previous agricultural land in Eastern Europe on the habitat selection and distribution of robo- and TBE- carrying rodents	ROBO 4	20	60 In progress	METLA
D ROBO 19	EDEN Robo documents on field sampling and animal ethics in wild rodent research	ROBO 4	17	60 In progress	METLA
D ROBO 20	Result of the shedding patterns of PUUV in nature	ROBO 4	60	60 In progress	METLA
D ROBO 21	Detailed results on the rodent-borne virus and host genetic variation in the course of strong rodent host fluctuations	ROBO 4	60	60 In progress	METLA
D LEI 1	Standardized spatial GIS data base of environmental and climate descriptors for 3 study regions (raw data sets – processed data-layers).	LEI 1	12-15	60 In progress	NHM/ LSHTM
D LEI 2	Standardized descriptions and maps (GIS models) of environments and climates in 3 study regions.	LEI 1	18	60 In progress	NHM/ LSHTM
D LEI 3	Standardized spatial data base of sandfly species in each study region.	LEI 2	15	60 In progress	NHM

D LEI 4	Standardized maps of distributions of sandfly species in each study region.	LEI 2	18	60 In progress	NHM
D LEI 5	Report on molecular findings, including conclusions on population structure and dispersal in relation to environmental variation in the adjacent study regions in Catalonian Spain and southwest France.	LEI 2	18	67 In progress	NHM
D LEI 6	Standardized spatial and temporal databases of *Leishmania* species and strains isolated from patients (classed by sex and age etc) in all 9 study regions.	LEI 3	15	60 In progress	UM1
D LEI 7	Descriptive analysis of spatial & temporal changes of prevalence rates in all 9 regions in the past 20 years.	LEI 3	18	67 In progress	UM1
D LEI 8	Inception of an European initiative to standardize national surveillance systems and networks.	LEI 3	18	67 In progress	UM1
D LEI 9	Standardized spatial and temporal databases of *Le. infantum* strains isolated from dogs (classed by sex, age, "occupation" etc) in all 9 study regions.	LEI 4	15	18	UB
D LEI 10	Descriptive analysis of spatial changes of prevalence rates in all 9 study regions in the past 20 years.	LEI 4	18	67 In progress	UB
D LEI 11	Inception of an European initiative to standardize national surveillance systems and networks.	LEI 4	18	67 In progress	UB
D LEI 12	The first preliminary model for 3 European regions of the spatial & temporal associations between leishmaniasis prevalence, its vectors, and changes in climate & environmental descriptors.	LEI 5	18	60 In progress	LSHTM
D LEI 13	Standardized spatial GIS data base of environmental and climate descriptors for all study regions (raw data sets – M24, processed data-layers – M30).	LEI 1	24-30	54 In progress	NHM
D LEI 14	Standardized descriptions and maps (GIS models) of environments and climates in all study regions.	LEI 1	30	54 In progress	NHM
D LEI 15	Standardized spatial data base of new records of sandfly species in each study region.	LEI 2	30	60 In progress	NHM
D LEI 16	Preliminary analysis of spatial & temporal changes of prevalence rates in some regions in the past 20 years.	LEI 3	30	60 In progress	UM1
D LEI 17	Preliminary analysis of spatial & temporal changes of incidence rates in some regions in the past 20 years.	LEI 4	30	60	UB
D LEI 18	Report on new prospective studies in southern France, northern Spain and Italy.	LEI 4	30	44 Done	UB
D LEI 19	The first preliminary model for all regions of the spatial association between leishmaniasis prevalence, its vectors, and climate & environmental descriptors.	LEI 5	30	60 In progress	LSHTM
D WNV 1	Document with preliminary description of study areas and practical information for decisions on study sites and study routines.	WNV 1	4	30 Done	EID
D WNV 2	Report of group-discussion to standardize approaches.	WNV 1	4	4 Done	EID
D WNV 3	Operational field and lab equipment.	WNV 1	4	4 Done	EID
D WNV 4	Summary reports on seasonal profiles of ornithophilic, horse-biting and human-biting mosquito species based on monthly reports.	WNV 2	12-18	30 Done	IPP
D WNV 5	Summary records of short-term variations in biting behaviour coupled with records of short-term variations in climate variables (i.e. weather).	WNV 2	12-18	48	IPP
D WNV 6	Preliminary list of ectoparasites associated with bird species. resident and migratory.	WNV 2	12-18	60 In progress	IPP
D WNV 7	PCR results for WNV in bird ectoparasites for sero-negative and seropositive birds.	WNV 2	18	60 In progress	IPP
D WNV 8	PCR results for WNV in over-wintering Culex pipiens populations.	WNV 2	18	60 In progress	IPP

D WNV 9	Preliminary data on survival rate of over-wintering Cx pipiens.	WNV 2	18	60 In progress	IPP
D WNV 10	Review of historical information on incidence/prevalence of WNV in humans and horses.	WNV 3	12-18	12	IVB
D WNV 11	Reports on seroprevalence of WN antibodies by species, sex, age, habitat and season.	WNV 4	12-18	48 Done	All teams
D WNV 12	Reports on seroprevalence and sero-conversion rates in horses.	WNV 4	12-18	67 In progress	All teams
D WNV 13	Preliminary data and analysis of seasonal variations in composition and abundance of the avifauna in the Rhone delta and other selected areas.	WNV 4	12-18	48 Done	All teams
D WNV 14	Preliminary data and analysis of the role of birds in introduction and diffusion of the West Nile virus (revised deliverable)	WNV 4	42	67 In progress	All teams
D WNV 15	Preliminary epidemiological analysis of the first field study season.	WNV 5	18	60 In progress	IPP, CIRAD, IZS
D WNV 16	Comprehensive data set in a common format.	WNV 1	26	67 In progress	EID
D WNV 17	An estimate of flavivirus seroprevalence in the human population.	WNV 3	30	Abandoned	IVB
D WNV 18	Intensive collections of mosquitoes for at least 10 consecutive nights (two weeks) at three points during the mosquito season by CO2-baited and bird-baited traps.	WNV 2	M36	M48	IPP
D WNV 19	Aspirator collections of mosquitoes attracted to horses during mosquito collection weeks.	WNV 2	M36	M48	IPP
D WNV 20	Collections of ectoparasites associated with birds-resident and migratory-captured for serosamples.	WNV 2	M40	M48	IPP
D WNV 21	Capture-mark-release of over-wintering Culex pipiens populations.	WNV 2	M37-40	M47-51	IPP
D WNV 22	Screen tests for virus in mosquito and ectoparasite collections by RAMP; confirmation of positives by virus isolation.	WNV 2	M41	M48	IPP
D WNV 23	A complete database on biotopes and habitats collected during the project, plus a compendium on historical material where this is of interest.	WNV1	60	67 In progress	All teams
D WNV 24	Article that includes seasonal fluctuations of all species captured	WNV 2	67	In progress	All teams
D WNV 25	Publications describing findings of WP4	WNV 4	67	In progress	All teams
D WNV 26	Publications describing findings of WP5	WNV 5	67	In progress	All teams
D MAL 1	Report of environmental parameters linked to anopheles vectors and malaria parasites.	MAL 1	12	60 In progress	All partners
D MAL 2	Spatial database on environment in every selected area.	MAL 1	12	60 In progress	All partners
D MAL 3	Standardized description of environment and climate into the model areas linked to anopheles vectors and malaria parasites.	MAL 1	18	60 In progress	All partners
D MAL 4	First draft of environmental map of model areas related to anopheles breeding sites.	MAL 1	18	60 In progress	EID
D MAL 5	Report of past and current anopheline species in selected model areas and in every partner country.	MAL 2	12	42 Done	All teams
D MAL 6	Database on anopheline collections in model areas.	MAL 2	48	54 In progress.	All teams
D MAL 7	Report and articles on biology and vectorial role of anopheline populations from model areas.	MAL 2	18	48 Done	All teams

D MAL 8 (updated)	Papers on anopheline systematics and population genetics.	MAL 2	18	67 In progress	All teams
D MAL 9 (updated)	Papers on vector competence.	MAL 2	18	67 In progress	IRD
D MAL 10	Report on current and past autochthonous and imported malaria in all 8 "field" country.	MAL 3	12	42 Done	IHMT
D MAL 11	Report on human movements in relation to malaria importation/exportation in all 8 "field" country, and particularly in model areas.	MAL 3	18	67 In progress	IHMT
D MAL 12	Report on the relationship between modifications of environment/human activities and malaria/potential vectors in model areas.	MAL 3	18	67 In progress	IHMT
D MAL 13 (updated)	Geo-referenced database on environment, vectors, malaria, and public health.	MAL 5	18	60 In progress	IRD
D MAL 14 (updated)	Maps and risk models in selected areas.	MAL 5	18	67 In progress	IRD
D MAL 15	Common bibliographic database.	MAL 5	30	60 In progress	IRD
D AFR 1	Census of RVF outbreaks since the 80's in the region, with available information on detailed location, date, etc.	AFR 1	8	38 Done	ISRA
D AFR 2	Description of WNF distribution in poultry and horses in the Senegal River delta and valleys	AFR 1	18	54 In progress	ISRA
D AFR 3	Map with selected study areas	AFR 1	18	38 Done	ISRA, CIRAD
D AFR 4 (updated)	Spatial database of environmental information available from each study area in Senegal	AFR 1	16	48 Done	ISRA, CIRAD
D AFR 5	Analysis of mosquitoes and ectoparasites census data in relation to their potential role in RFV epidemics	AFR 2a	18	67 In progress	IPD
D AFR 6	Comparative study on the blood-feeding entomo-fauna in disease foci and in the northern potential sites	AFR 2a	18	67 In progress	IPD
D AFR 7	Rearing of first candidate mosquitoes	AFR 2a	18	37 Done	IPD
D AFR 8	Report on the vectorial capacity of local and exotic species	AFR 2a	18	Not achievable	IPD
D AFR 9	Description of the impact of some abiotic factors on the vectorial capacity	AFR 2a	18	Not achievable	IPD
D AFR 10	Report (list and census) on the migrant and resident wild birds species in selected areas	AFR 2b	18	Not achievable	IRD
D AFR 11	Report on the WNV seroprevalence in wild birds of Senegal and Morocco	AFR 2b	18	60 In progress	IRD, IPD
D AFR 12	Comparative between-site study report on the candidate WNV vector mosquito species	AFR 2b	18	67 In progress	IRD, IPD, ISRA
D AFR 13	Study report on the links between RVF cases and foci at the Senegal river scale	AFR 4	18	38 Done	ISRA, IPD

D AFR 14	Preliminary spatial information system and protocols for data management	AFR 5	18	36 Done	CIRAD
D AFR 15	Extension of census of RVF outbreaks since the 80's in the region (Mali, Mauritania).	AFR 1	30	48 Done	ISRA
D AFR 16 (updated)	Landscape typology in Senegal.	AFR 1	30	39 Done	ISRA, CIRAD
D AFR 17 (updated)	Description of major livestock farming systems associated to landscapes in Senegal.	AFR 1	30	48 Done	ISRA, CIRAD
D AFR 18	Report on WN infected mosquitoes and ectoparasites.	AFR 3	30	60 In progress	IPD, IRD, ISRA
D AFR 19	Isolation of WNV strains in Africa.	AFR 3	30	38 Done	IPD
D AFR 20	Virus variability between foci (spatial and temporal) for RVF and WNF.	AFR 4	30	60 Done	IPD
D AFR 21	Description of regional (ruminants, birds) and transcontinental (birds) movements of hosts and reservoirs.	AFR 4	30	67 In progress	ISRA, IRD, CIRAD
D AFR 22	First attempts for spreading models.	AFR 4	30	60 In progress	ISRA, CIRAD
D AFR 23	Integration of remote sensing data for monitoring of key environmental parameters.	AFR 5	36	48 Done	CIRAD
D AFR 24	Importation of the available field data and remote sensing data	AFR 5	30	48 Done	CIRAD
D AFR 25	First reports of simulations in the conceptual models	AFR5	42	54 In progress	CIRAD
D DMT01	User questionnaires	6.1	3	3 Done	Euro-Aegis
D DMT02	Data standardisation guidelines	6.1	4	5 Done	Euro-Aegis
D DMT03	Data standardisation workshop and reports	6.1	5	5 Done	Euro-Aegis
D DMT04	Report on user needs and list of data needs for the first 18 months	6.1	6	6 Done	Euro-Aegis
D DMT05	IDEAS - Web based data archive software beta version	6.1	12	12 Done	Euro-Aegis
D DMT06	IDEAS - Web based data archive software final version	6.1	18	18 Done	Euro-Aegis
D DMT07	Data request lists	6.1	18	18 Done	Euro-Aegis
D DMT08	Networking at international workshops and conferences to assess EDEN external user needs	6.1	18-30-42-48	18-36-48-60-67 Done	Euro-Aegis
D DMT09	New epidemiological EDEN SP data sets, and new 'upon request' data sets from internal and external EDEN users are included in EDEN DMT website.	6.1	18-30-42-48	18-36-48-60-67 Done	Euro-Aegis

D DMT10	Provision of value added data processing to assist with interpretation of EDEN outputs	6.1	18-30-42-48	18-36-48-60-67 Done	Euro-Aegis
D DMT11	GIS module and exercises adapted to GIS software packages selected at AM.	6.1	30	30-42 Done	Euro-Aegis
D DMT12	Respective GIS training module manuals.	6.1	34	34-46 Done	Euro-Aegis
D DMT13	Operational PhD section.	6.1	36	36 Done	Euro-Aegis
D DMT14	Provision of on-demand training in Geographic Analysis, using manuals, online assistance or tailored courses, as appropriate.	6.1	18-30-42	18-36-48-67 Ongoing	Euro-Aegis
D DMT15	Contribution to discussion on EDEN disease information system at the EDEN SC and AG meetings, and meetings involving international user organizations.	6.1	18-30-42-48	18-36-48-60-67 Done	Euro-Aegis
D DMT16	ICT proposals for EDEN disease information system.	6.1	42	42 Done	Euro-Aegis
D DMT17	GIS module included in Moodle distance learning tool.	6.1	60	62 In progress	Euro-Aegis
D HRRS01	High resolution image analysis for each selected site.	6.2	24	36-60 In progress	UCL
D HRRS02	Landscape analysis and environmental change analysis for selected HR sites.	6.2	24	36-60-67 In progress	UCL
D HRRS03	Conceptual model for each disease under study in EDEN.	6.2	24	36-60-67 In progress	UCL, CIRAD
D HRRS04	On the job HRRS technology transfer.	6.2	24	24-36-48 Done	UCL
D LRRS01	Fourier processed MODIS data archive.	6.2	18	24-36-48-60 In progress	ZOOX
D LRRS02	Pan-European maps of eco-climatic seasonality signals.	6.2	24	60 In progress	ZOOX
D LRRS03	Pan-European maps of space-time environmental changes.	6.2	24	36 Done	ZOOX
D LRRS04 (updated)	Information-theoretic models of identified risk factors for selected diseases.	6.2	30	36-60-67 In progress	ZOOX
D LRRS05	On the job LRRS technology transfer	6.2	24	36-48-60 In progress	ZOOX
D LRRS06	First process-based vector models for candidate diseases	6.2	30	60 In progress	ZOOX
D LRRS 07	Data-fusion techniques for selected imagery developed	6.2	36	60 Done	ZOOX
D LRRS 08	Analysis of changes detected through moving windowed Fourier techniques	6.2	36	67 In progress	ZOOX
D PhD 01	Respective methodologies included in strategy document.	6.3	18	24 Done	UCL-FVM-IRD
D PhD 02	Respective PhD progress reports.	6.3	24	24 Done	UCL-FVM-IRD
D OI01	Strategy document v 2.0	7	24	24 Done	CIRAD ZOOX

D OI02 (changed to D 0I05)	Inputs from SC, AG members, selected international users and the EDEN PhD network members are included in the EDEN strategy document v2.1. (M34).	7	24	34	CIRAD
D OI03 (changed to D 0I05)	Strategy document v3.0 – includes chapters on disease specific models provided by EDEN SP leaders (M39).	7	30	39	CIRAD
D OI04 (changed to D 0I05)	Inputs from SC, AG members, selected international users and the EDEN PhD network members are included in the EDEN strategy document V3.0 (M46)	7	30	36	CIRAD
D OI05	The strategy document is published as the proceedings or an edited book after the International EDEN conference at the end of the very last reporting period.	WP 7		67	CIRAD, ZOOX, Euro-Aegis
D MAN01	EDEN second annual report	WP 7	25	25 Done	CIRAD
D MAN02	Second Annual EDEN PhD network meeting	WP 7	27	27 Done	CIRAD
D MAN03	EDEN AGM2 in Antalya, Turkey	WP 7	27	27 Done	CIRAD
D MAN04	EDEN SC and AG meeting at AGM2	WP 7	27	27 Done	CIRAD
D MAN05	EDEN Newsletter 6 - AGM presentations	WP 7	30	34 Abandoned	CIRAD
D MAN06	Mandatory report on raising public participation and awareness	WP 7	30	42 Done	CIRAD
D MAN07	Mandatory report on gender action	WP 7	30	60 In progress	CIRAD
D MAN08	EDEN SC meeting in London	WP 7	32	32 Done	CIRAD
D MAN09	EDEN Newsletter 7 – Publications	WP 7	34	38 Abandoned	CIRAD
D MAN10	EDEN SC meeting in Brussels	WP 7	36	36 Done	CIRAD
D MAN11	EDEN third annual report	WP 7	38	Done	CIRAD
D MAN12	Third Annual PhD network meeting	WP 7	39	Done	CIRAD
D MAN13	Annual meeting AGM3 in Brno Czech Republic	WP 7	39	Done	CIRAD
D MAN14	EDEN SC and AG meeting at AGM3	WP 7	39	Done	CIRAD
D MAN15	EDEN Newsletter 8 - Publications update	WP 7	42	Abandoned	CIRAD
D MAN16	New version of EDEN poster and leaflet (as a replacement of EDEN newsletters)	WP 7	42	Done	CIRAD
D MAN17	List of EDEN publication	WP 7	48-67	Done	CIRAD, Avia-GIS
D MAN18	EDEN SC in Paris	WP 7	44	Done	CIRAD, Avia-GIS
D MAN19	EDEN fourth annual report	WP 7	48	Done	CIRAD, Avia-GIS
D MAN20	Fourth Annual PhD network meeting	WP 7	49	Done	CIRAD, Avia-GIS
D MAN21	Annual meeting AGM4 in Marrakesh, Morocco	WP 7	49	Done	CIRAD
D MAN22	EDEN SC and AG meeting at AGM4	WP 7	49	Done	CIRAD, Avia-GIS

D MAN24	EDEN SC in UK	WP 7	54	26-27Oct	CIRAD, Avia-GIS
D MAN25	EDEN SC in Brussels	WP 7	60	To be agreed	CIRAD, Avia-GIS
D MAN26	Final steering committee meeting in Montpellier	WP 7	67	In progress	CIRAD, Avia-GIS
D MAN27	Final international conference in Montpellier	WP 7	67	In progress	CIRAD, Avia-GIS
D MAN28	Final report	WP 7	67	In progress	CIRAD, Avia-GIS

Table 2: Milestones List

List all milestones, giving date of achievement and any proposed revision to plans.

Milestone no.	Milestone name	Workpackage no.	Date due	Actual/Forecast delivery date	Lead contractor
M TBD 01	National database of standardized climatic data for each TBD partner country at the national level to reveal the quality of the data upon which multi-national databases can be built.	TBD 1	3	12 Done	ZOOX
M TBD 02	Epidemiological information from WP3 is available and allows the selection of areas for high-resolution land cover/land use change studies	TBD 1	6	12 Done	ZOOX
M TBD 03	Laboratory staffs of national TBD teams are trained in using newly developed advanced molecular techniques	TBD 2	15	24 Done	LMU
M TBD 04	Deployment of trained (standardised field sampling protocols) staff to cover each site	TBD 2	16	24 Done	LMU
M TBD 05	The first samples to indicate the feasibility, suitability and sustainability of the sampling regime	TBD 2	17	24 Done	LMU
M TBD 06	The first molecular diagnostic data to indicate the sensitivity and specificity of the methods.	TBD 2	20	24 Done	LMU
M TBD 07	Spatial patterns in epidemiological data and in changes in climate and land cover/land use (WP1) will direct the further selection of particular indices of sociological and behavioural change that may have increased human contact with infected ticks.	TBD 3	14	14 Done	PHA
M TBD 08	Identification of tick-infested areas with known variable roe deer densities to inform the positioning of trapping grids	TBD 4	13	13 Done	CEA
M TBD 09	Samples of rodents from the first five months of trapping indicate any need for changes in sampling and processing protocols.	TBD 4	21	21 Done	CEA
M TBD 10	Definition of structure and components of database appropriate for the nature of the information (quantitative, qualitative, discrete and continuous).	TBD 5	30	38 In progress	ZOOX
M TBD 11	Evidence that changes in public health do not account for the historical epidemiological patterns and therefore environmental or sociological causes are more likely.	TBD 5	24	24 Done	ZOOX
M TBD 14	The first molecular diagnostic data to indicate the sensitivity and specificity of the methods.	TBD 2	30	34 Done	ZOOX
M TBD 15	Spatial patterns in sociological factors and land cover/land use (WP1) in relation to epidemiological data will direct the further selection of particular indices of sociological and behavioural change that may have increased human contact with infected ticks .	TBD 3	36	36 Done	ZOOX
M TBD 16	First results of serological evidence of infection in rodents with TBE virus.	TBD 4	30	30 In progress	CEA
M TBD 18	The spatial database on climate and land cover/land use changes is periodically updated.	TBD 1	36-48	48 Done	ZOOX
M TBD 19	The first data to indicate the sensitivity and specificity of the methods of blood meal analysis.	TBD 2	42	42 Done	LMU / Neuchâtel

M TBD 20	Trial indices of deer abundance at selected sites	TBD 4	42	42 Unlikely	CEA
M TBD 21	Field sites for rodent trapping in Latvia will be identified.	TBD 5	42	42 Done	ZOOX / ROBO
M ROBO 1	Trainers are trained and disseminate their knowledge.	ROBO 1	4	4 Done	UA
M ROBO 2	Raw data are collected to create spatial data layers.	ROBO 1	8	18 Done	UA
M ROBO 3	Spatial data layers are used to generate spatial analysis models.	ROBO 1	12	24 In progress	UA
M ROBO 4	Model outputs are available and allow for (i) multi-factorial spatial analysis on virus/rodent dynamics and (ii) extrapolation to other parts of Europe at later stage of project (WP5).	ROBO 1	18	26 In progress	UA
M ROBO 5	The dissemination of the standardised human diagnostics protocol and the training of personnel allow standardised sampling and processing throughout Europe.	ROBO 3	6	6 Done	UH.HI
M ROBO 6	First and second generation maps of observed human roboviral presence in Europe allow for fine tuning of the human sampling campaign(s).	ROBO 3	8-18	20-30 Done	UH.HI
M ROBO 7	Sero-data, especially those from groups at highest risk, contribute to target work in WP 4.	ROBO 3	12-18	13-30 Done	UH.HI
M ROBO 8	The dissemination of the various standardised rodent sampling and viral diagnostics protocol and the training of personnel allow standardised sampling of data at the various required scales throughout Europe.	ROBO 4	6	6-12 Done	METLA
M ROBO 9	First and second generation maps of observed rodent and roboviral reservoir presence in Europe allow for fine tuning of the sampling campaign(s): monitoring network and cross-sectional sampling.	ROBO 4	12-18	13-30 Done	METLA
M ROBO 10	Relevant spatial data are made available to include in GIS model.	ROBO 5	12	30 Done	UA
M ROBO-11	Environmental data used for understanding the distribution of rodent-borne viruses and their hosts as well as their dynamics and epidemiology	ROBO 1	M36	M60 In progress	ZOOX, EURO-AEGIS
M LEI 1	First workshop for teams involved with initial 3 study regions, to provide training for GPS ground mapping of environments and construction of data layers. Workshop will serve as the control point for deciding the precise areas to be mapped in each study region. To be held before the first leishmaniasis transmission season (May-October).	LEI 1	4	4 Done	NHM
M LEI 2	Collected raw data (including fieldwork in summer 2005) used to generate spatial data layers for each of two time points (c. 1980 & most recent). -	LEI 1	12	12 Done	NHM
M LEI 3	Spatial data layers used to generate preliminary spatial analysis models	LEI 1	15	15 Done	NHM
M LEI 4	Second workshop for teams involved with initial 3 study regions to meet other EDEN-LEI teams, to decide the extent of similar work in the other 6 regions later in the project	LEI 1	15	16 Done	NHM
M LEI 5	Month 18. Preliminary spatial model outputs are available and allow for 1) first spatial analysis of vector/ disease/ environment associations in 3 study regions (WP5), and 2) extrapolation to other regions at a later stage	LEI 1	18	18 In progress	NHM
M LEI 6	First workshop for all teams, to provide training for data basing and construction of data layers. Workshop will serve as the control point for deciding the precise areas to be mapped in each study region. To be held before the first leishmaniasis transmission season (May-October)	LEI 2	4	4 Done	NHM
M LEI 7	Collected raw data (with new data from summer 2005) used to generate spatial data layers for each region	LEI 2	12	24 Done	NHM

M LEI 8	Spatial data layers used to generate preliminary spatial analysis models.	LEI 2	15	48 In progress	NHM
M LEI 9	First workshop for all teams, to provide training for data basing and construction of data layers	LEI 3	4	4 Done	UM1
M LEI 10	Second workshop for all teams, to try to standardize the national systems for surveillance of human leishmaniasis and the method of molecular characterization of Leishmania	LEI 3	4	4 Done	UM1
M LEI 11	Collected raw data used to generate spatial data layers for each of two time periods (pre-1980, more recent)	LEI 3	12	15 Done	UM1
M LEI 12	Month 15. Spatial data layers used to generate preliminary spatial analysis models	LEI 3	15	18 In progress	UM1
M LEI 13	First workshop for all teams, to provide training for data basing and construction of data layers	LEI 4	4	4 Done	UB
M LEI 14	Second workshop for all teams, to try to standardize the national systems for surveillance of canine leishmaniasis and the method of molecular characterization of Leishmania	LEI 4	4	4 Done	UB
M LEI 15	Collected raw data for 3 detailed study regions used to generate spatial data layers for each of two time periods (pre-1980, more recent	LEI 4	12	12 Done	UB
M LEI 16	Spatial data layers used to generate preliminary spatial analysis models.	LEI 4	15	50 In progress	UB
M LEI 17	Preliminary spatial model outputs are available and allow for 1) first spatial analysis of disease/ vector/ environment associations in 3 detailed study regions (see WP1), and 2) extrapolation to other regions at a later stage	LEI 4	18	60 Just started	UB
M LEI 18	Collected raw data (including fieldwork in summer 2005) used to generate spatial data layers for each of two time points (c. 1980, most recent)	LEI 4	12	30 Done	UB
M LEI 19	Spatial data layers used to generate preliminary spatial analysis models	LEI 5	15	50 In progress	LSH&TM
M LEI 20	Preliminary spatial model outputs are available and allow for 1) first spatial analysis of vector/ disease/ environment associations in 3 detailed study regions, and 2) extrapolation to other regions at a later stage	LEI 5	18	50 In progress	LSH&TM
M LEI 21	Workshop to update all EDEN-LEI teams	LEI 5	18	30 Done.	NHM
M LEI 22	Training workshop in Rovaniemi (EDEN AGM) for eastern teams. With the assistance of all western teams, this will decide the extent of WP1 work in the eastern regions.	LEI 1-5	15	15 Done	NHM
M LEI 23	Training workshop in Montpellier for French and northern Spanish teams.	LEI 1-5	17	17 Done	NHM
M LEI 24	SP co-ordinator (Dr Ready) and PhD student provide one-on-one training for eastern teams during short visits to field sites in Greece, Hungary and Turkey.	LEI 1-5	21	19 Done	NHM
M LEI 25	Report on preliminary analysis of spatial & temporal changes of prevalence rates in some regions in the past 20 years.	LEI 3	30	48 Done	UM1
M LEI 26	Preliminary spatial model outputs are available and allow for extrapolation to all regions.	LEI 5	30	60 In progress	NHM
M LEI 27	To agree deadlines for D7, D8, D16	LEI 3	37	52 In progress	NHM
M LEI 28	To monitor progress for D7, D8, D16	LEI 3	31	55 In progress	NHM
M LEI 29	To agree deadlines for D10, D11	LEI 4	42	55 In progress	NHM
M LEI 30	To monitor progress for D10, D11	LEI 4	29	55 In progress	NHM

M LEI 31	Training workshop (hopefully in Granada) for all teams	LEI 5	42	44 Done	NHM/UB
M WNV 01	Unify partners as a pan-European group: exchange ideas, generate enthusiasm, promote dialogue	WNV 1	5	12 Done	IPP & all teams
M WNV 02	Define study sites in study areas	WNV 1	5	5 Done	IPP & all teams
M WNV 03	Agree on standardized common protocol	WNV 1	5	24	IPP & all teams
M WNV 04	Prepare all field and laboratory equipment	WNV 1	5	24 Done	IPP & all teams
M WNV 05	Host preference and seasonality of mosquitoes in study areas	WNV 2	18	54 In progress	IPP & all teams
M WNV 06	Effect of short-term variations in climate on abundance and behaviour of mosquitoes in study areas will give clues as to likely impacts of long term climate change on transmission	WNV 2	18	54 In progress	IPP & all teams
M WNV 07	Evidence for/against role of ectoparasites in WNV transmission dynamics	WNV 2	18	60 In progress	IPP, Romania, IZS
M WNV 08	If there is evidence of current WNV transmission (seroconversion in birds, or horse or human cases), pools of mosquitoes will be tested for virus	WNV 2	18	48 Done	IPP
M WNV 09	Winter survival rates of mosquitoes and WN minimum infection rates will be key parameters in models of epizootic transmission	WNV 2	18	54 In progress	IPP
M WNV 10	Data collected in the first months of trapping will contribute to epidemiological modelling and analysis	WNV 2	10	60 In progress	IPP
M WNV 11	Information will contribute to epidemiological modelling and analysis	WNV 3	10	60 In progress	IVB
M WNV 12	Data over several seasons will indicate principal species that appear to be involved in transmission, and seroconversion rates of juveniles	WNV 4	18	48 Done	All teams
M WNV 13	Comparison of species data with mosquito capture data within and between study regions will build a picture of the bird/mosquito species involved in transmission, the habitats/mosquitoes associated with this transmission, and regional differences in transmission dynamics	WNV 4	18	60 In progress	All teams
M WNV 14	Seroprevalence and seroconversion rates, combined with seasonal information from mosquito captures, will contribute to understanding of seasonality of transmission, which in turn may help interpret impact of variations between seasons, and indicate likely impacts of future climate change	WNV 4	18	60	All teams and HITs
M WNV 15	Seroprevalence in migratory birds will indicate species that are likely to be involved in intercontinental transport of virus, and may provide clues as to contact points between migratory and resident species	WNV 4	18	60	All teams and HITs
M WNV 16	Data collected during the first seasons will contribute to epidemiological modelling and analysis M18	WNV 4	18	48 Done.	IVB
M WNV 17	Data collected during in the first 24 months will contribute to epidemiological modelling and analysis	WNV 5	18	60 In progress	IPP
M WNV 18	Completed dossiers of data and other information, and a comprehensive data set in a format that is common to all contractors.	WNV 1	26	54 In progress	EID
M WNV 19	An assessment of risk of infection in humans in areas where transmission is known to occur	WNV 3	48	Done	IZS, NIRDMIC
M WNV 21	A completed foundation for all milestones in WP 5, plus a publication on history of WNV in Romania (NIRDMIC). This will be the basis of a publication on the history of WNV worldwide.	WNV1	60	60	NIRDMIC

M MAL 1	List of precise environmental parameters to be recorded in every model area	MAL 1	3	3 Done	EID
M MAL 2	Workshop on environment, data management and sampling	MAL 1	6	6 Done	EID
M MAL 3	Detailed environmental information about model areas	MAL 1	12	24 Done	EID
M MAL 4	Collected raw data (including archived data and fieldwork in spring-summer 2005) to create spatial data layers	MAL 1	12	48 In progress	EID
M MAL 5	Preliminary spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the 8 model areas	MAL 1	18	48 In progress	EID
M MAL 6	List of precise biological parameters to be recorded in every model area	MAL 2	3	3 Done	NIRDMI
M MAL 7	Workshop on sampling	MAL 2	6	4 Done	NIRDMI
M MAL 8	Collected raw data (including archived data and fieldwork in spring-summer 2005) to create anopheline data layers	MAL 2	12	24 Done	NIRDMI
M MAL 9 (updated)	Spatial model of vector/ malaria/ environment associations in the 8 model areas	MAL 2	18	60 In progress	All teams
M MAL 10	Data on past and present malaria in the 8 "field" countries	MAL 3	12	18 Done	IHMT
M MAL 11	Data autochthonous and imported malaria incidence in the 8 "field" countries, including data from model areas	MAL 3	12	54 In progress	IHMT
M MAL 12	Collected raw data to create public health – human activities layers	MAL 3	12	54 In progress	IHMT
M MAL 13 (updated)	Spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the 8 model areas	MAL 3	18	60 In progress	IHMT
M MAL 14 (updated)	Spatial model of vector/ malaria/ environment associations in the 8 model areas	MAL 5	18	60 In progress	IRD
M MAL 15	Workshop on data management and GIS	MAL 5	18	24 Done	IRD
M MAL 16	Development of a common database to all malaria SP partners	MAL 5	18	54 In progress	IRD
M MAL 17	Collected raw data related to second model area are available.	MAL 1	36	48 In progress	EID
M MAL 18	Detailed environmental information about second model areas is available.	MAL 1	30	48 In progress	EID
M MAL 19	Biology and distribution of *An. Plumbeus*.	MAL 2	24	48 Done	NIRDMI
M MAL 20	Infection rate of natural anopheline population in model areas in Algeria, Morocco and Turkey.	MAL 2	24	54 In progress	Turkey
M MAL 21	Vector competence of at last 6 European populations of Anophelines.	MAL 2	24	60 In progress	NIRDMI
M MAL 22	First results on population genetics of *An. Maculipennis*.	MAL 2	30	54 In progress	NIRDMI
M MAL 23	Update of distribution of Anopheline species in partner countries.	MAL 2	30	54 In progress	NIRDMI
M MAL 24	Data on human population at risk of reservoir	MAL 3	30	30 Done	IHMT
M MAL 25	Data on human behavior related to mosquito and malaria	MAL 3	24	42	IRD
M MAL 26	First scientific publications on mosquito biology in international journal	MAL 2	24	48 Done	IRD
M MAL 27	First scientific publications on modelling in international journal	MAL 5	36	42 Done	IRD
M AFR 01	Field survey results enable to produce the map of WNF distribution in Senegal River delta and valley	AFR 1	18	60 In progress	ISRA
M AFR 02	The serological analysis have been conducted and data are available for analysis of patterns	AFR 1	18	60 In progress	ISRA

M AFR 03	Regional database on RVF events in domestic ruminants in Mali, Mauritania and Senegal is on the EDEN Web-site	AFR 1	18	60 In progress	ISRA
M AFR 04	Regional database on RVF events in domestic ruminants in Mali, Mauritania and Senegal is on the EDEN Web-site.	AFR 2a	6	60 In progress	ISRA
M AFR 05	The list of candidate arthropods for RVF transmission has been up-dated.	AFR 2a	6	60 No infection found	IRD
M AFR 06	New mosquito colonies have been established.	AFR 2a	18	36 Done	IPD
M AFR 07	First experimental mosquito infections and host inoculations have been initiated.	AFR 2a	18	42 Unlikely	IPD
M AFR 08	A report on vectorial capacity of candidate vector mosquitoes is available.	AFR 2a	18	42 Unlikely	IPD
M AFR 09	Updated bird census is available.	AFR 2b	18	38 Unlikely	IRD
M AFR 10	Blood collections of birds have been initiated.	AFR 2b	18	38 Done	IRD
M AFR 11	Serological analyses have been conducted and prevalence data in resident and migratory wild birds are available.	AFR 2b	18	30 Done, not available	IRD, IPD
M AFR 12	WN virus strains have been isolated.	AFR 2b	30	36 Done, not available	IPD
M AFR 13	Potential explicative parameters of the relationship between foci in Senegal and Mauritania are identified and mapped	AFR 4	18	60 In progress	IPD
M AFR 14	A dendrogram for RVFV and WNV is drawn and describes the genetic similarities between strains from various regional areas	AFR 4	30	38 Done for RVF	IPD
M AFR 15 (updated)	Ruminant densities and movements in Sub-Saharan Africa.	AFR 4	30	60 in progress	ISRA, CIRAD
M AFR 16	The conceptual model is validated and the database organised for implementation	AFR 5	18	60 In progress	CIRAD
M AFR 17	The GIS is developed and include the metadata of the conceptual model	AFR 5	30	60 In progress	CIRAD
M DMT01	EDEN SP epidemiological data made available.	6.1	14	60 In progress	Euro-AEGIS
M DMT02	External EDEN users respond to questionnaire.	6.1	12-24	12-24 Done	Euro-AEGIS
M DMT03	EDEN partners request DMT training input.	6.1	12-24-36-48	12-24-36-48-60 Done	Euro-AEGIS
M DMT04	International workshops and conferences are identified to enable proper networking and identification of external user needs.	6.1	12-24-36-48	12-24-36-48-60 Done	Euro-AEGIS
M DMT05	Blueprint of EDEN PhD section of EDEN DMT website is available for discussion at EDEN PhD workshop.	6.1	27	27 Done	Euro-AEGIS
M DMT06	EDEN PhD network representatives are elected and actively contribute to the development of the EDEN PhD section of the EDEN DMT web.	6.1	27	27 Done	Euro-AEGIS
M DMT07	ICT proposals for EDEN disease information system are available to start the development of disease information system tools.	6.1	42	60 In progress	Euro-AEGIS
M DMT08	Partners contribute necessary information to develop 'eco-system' part of information system	6.1	60	60 In progress	Euro-AEGIS

M RS 01	MODIS raw data archive is available for data download.	6.2	12-18	From 30 Done	ZOOX
M RS 02	Downloaded MODIS data are Fourier processed and available to be included in spatial models.	6.2	18	From 36 Done	ZOOX
M RS 03 (updated)	The final format of the strategy document will be the proceedings of the EDEN International Conference to be held in May 2010 at the end of the requested 7 month no-cost extension.	6.2	18	67	ZOOX, EURO-AEGIS, CIRAD
M RS 04	Conceptual models for selected diseases enable to relate epidemiological variables with HRRS landscape analysis.	6.2	24	42	UCL
M RS 21	Synthesis of empirical findings on linkages between the different agents responsible for disease transmission (vectors, hosts, humans, environment)	6.2	21	1-67 Papers, incl. special issue Rev. Sci Tech. OIE	All EDEN partners
M RS 22	Integration of several HITs and selected SPs to leverage EDEN's results to maximum effect.	6.2	48-67		All EDEN partners
M RS 23	Development of a post-EDEN strategy for EDEN activities, results etc., especially concerning the future of EDEN's databases	6.2	48-67	48-67	All EDEN partners
M PhD 01	The discussions at the annual meeting enable to decide about respective collaborations with vertical sub projects and to write the PhD methodology.	6.3	15	15 Done	UCL-FVM-IRD
M PhD 02	The discussions at the RTD integration meeting enable to fine-tune approaches, proceed with PhD work and strengthen collaborations between post-graduates coming from various scientific backgrounds in order to reach a common goal.	6.3	18	18 Done	UCL-FVM-IRD
M OI 01	SC and AG members comment on the strategy document v1.0.	7	12-15	15 Done	CIRAD ZOOX
M OI 02	SC and AG members comment on the strategy document v2.0.	7	15-18	32 Done	CIRAD ZOOX
M OI 03	Strategy document v2.1 is available for comments by SC, AG members, selected international users and the EDEN PhD network.	7	27	27 Done	CIRAD
M MAN 01	The EDEN website offers a gateway to the general public and enables to reach an extra user group.	WP 8	15	15 Done	CIRAD
M MAN 02	The second annual meeting strengthens EDEN as a group and enables the establishment of new collaborations between partners.	WP 8	16	15 Done	CIRAD
M MAN 03	The RTD integration workshop establishes strong links between the EDEN PhD community.	WP 8	18	20 Done	CIRAD
M MAN 04	The EDEN Dissemination plan v2.0 takes into consideration EDEN's successes and failures in that field of activities and enables the implementation of an improved dissemination strategy.	WP 8	24	26 Done	CIRAD
M MAN 05	The second annual meeting strengthens EDEN as a group and enables the establishment of new collaborations between partners.	WP 8	27	27 Done	CIRAD
M MAN 06	The annual PhD network meetings strengthen links within the EDEN PhD community and promote scientific integration in EDEN.	WP 8	27	27 Done	CIRAD
M MAN 07	Inputs at SC and AG meetings enable in depth discussions on EDEN scientific strategy and contribute to problem identification and solving.	WP 8	27-32-36	27-32-36 Done	CIRAD
M MAN 08	Partners provide timely inputs for Newsletters.	WP 8	27-31-39	See NL item in text	CIRAD
M MAN 09	The updated EDEN Dissemination plan takes into consideration EDEN's successes and failures in that field of activities and enables the implementation of an improved dissemination strategy.	WP 8	38	38 Done	CIRAD
M MAN23	EDEN SC in UK. State of modelling work and preparation of the international conference	WP 8	56	56	CIRAD

M MAN24	EDEN SC in Brussels. State of modelling work and preparation of the international conference	WP 8	60	60	CIRAD
M MAN 25	Final EDEN poster and leaflet. Summary of EDEN results and advertisement for the EDEN international conference	WP 8	64	64	CIRAD
M MAN 26	Final steering committee meeting in Montpellier. Summary of EDEN results. Dissemination of EDEN results and appropriation by public-health agencies.	WP 8	67	67	CIRAD
M MAN 27	Final international conference in Montpellier	WP 8	67	67	CIRAD
M MAN 28	Final activity and management report.	WP 8	67	67	CIRAD

Section 3 – Consortium management

The EDEN management structure and its potential to solve problems

The official starting date of EDEN was the 1st of November, 2004. This date was chosen to fit the annual field sampling cycle and allows for a more convenient reporting and annual meeting cycle. However, the contract has been signed by the coordinator, following reception of all signed A-forms by the consortium partners, on the 3rd of December and by the EC on the 21st of December, 2004. Following this the EC contribution reached the coordinator on the 30th of December and most of the respective grants have been forwarded to all the EDEN partners during January 2005. Transfers at a later date were due to delays in signature of the Consortium Agreement by a minority of consortium partners and to the split of the Pasteur Institute Network in two distinct consortium partners (see below). Thus in practice, whilst preparatory activities already started end 2004 – early 2005, the operational start of EDEN was at the kick-off meeting in February 2005.

The central management team was operational from the onset of the project: Stéphane de La Rocque, project coordinator (replaced by Renaud Lancelot in January 2007), Guy Hendrickx, secretary of the Steering Committee, and Sylvie Pugin, administrative assistant on the 1st of November 2004 with timely back-up from Prof. David Rogers, chairman Steering Committee. Early January 2005, Olivier Pierre, financial officer, was recruited and joined the team. Prior to his arrival EDEN financial matters were taken care of by the CIRAD finance department. This central management team is in charge of day to day management and real time problem solving. A management handbook was disseminated and in order to facilitate the rapid flow of administrative and financial information the EDEN financial officer created a network of 'on the job' administrative and financial officers. All EDEN management team members respond within 24h to e-mail requests by EDEN consortium partners.

Because of the complexity of a project such as EDEN in addition to a strong central management team a compact Steering Committee (SC) of thirteen members was created, each member of the steering committee being, in addition to the project coordinator and the secretary of the SC, responsible for a well defined part of the project; either one of six vertical sub-projects (SP) devoted to the study of a specific disease group, or one of five horizontal integration activities (HIT). Thus each SC member is responsible for a well defined part of the Workplan, in permanent contact with her or his sub-project partners and permanently informed of the progress made by other teams. The SC meets three times per year alternatively in Paris, London or Brussels. This set-up proved to be very efficient and enabled EDEN to implement an efficient problem solving strategy. Most management problems were rapidly identified and solved.

Finally an external Advisory Group of ten members was established to guide scientific and technical decision making, mainly with regard to work programs beyond the first eighteen months. Whilst this was considered an important part of the EDEN management approach it proved difficult to implement. Currently Stéphane de La Rocque, previously EDEN coordinator, who left CIRAD to join FAO, has been appointed as chairman of the AG. His background and dynamism should de an asset. AG members have also been given specific tasks at the Annual meetings: chairing of plenary scientific sessions, election of the EDEN poster award, expert advice during round table discussions on risk mapping and disease information systems, and participation to the SC meeting. Whilst some AG members have little time to spare to participate to the EDEN AGM, individual AG members have showed interest to provide advice on demand during the year. The AG member list is (in alphabetical order):

- Stéphane De La Rocque, FAO EMPRES, Rome, Italy
- Bettina Menne, WHO-EU, Rome, Italy
- Duane Gubler, Asia-Pacific Institute for Tropical Medicine and Infectious Diseases, Honolulu, Hawaii

- Philippe Martin, EU DG-SANCO, Brussels, Belgium
- Santiago Mas Coma, Universitad de Valencia, Valencia, Spain
- Antonio Petrini, OIE, Paris, France
- Rainer Sauerborn, Universität Heidelberg, Heidelberg, Germany
- Jan Slingenbergh, FAO AGA, Rome, Italy
- Graham White, US Department of Agriculture, Gainesville, USA

The issue of external audit costs has been solved during year two. In order to reduce management costs, external audits at the time of the annual reports are only requested from partners who have declared more than € 150,000 expenditures in their (cumulated) C-forms during the previously non-audited time span.

Summary of the achieved management and coordination tasks

1. Permanent coordination tasks

The permanent coordination tasks are implemented on a daily basis by the EDEN management team. Scientific issues of general interest are discussed by the coordinator, the chairman of the SC and the secretary of the SC. If needed, proposed solutions are further discussed with SC members through e-mails or eventually at SC meetings. At the occasion of a training course given in Dakar (February 2008), the coordinator met the director of IPD (partner 48) and the representative of IRD (partner 13), and had a meeting with the African platform to find solutions to the data base / data-sharing issues.

The EDEN financial officer and administrative assistant are stand by on a permanent basis to respond relevant questions and contribute to problem solving. Financial monitoring is achieved through the network of financial officers of partner organizations. A monthly management meeting is held at CIRAD headquarters in Montpellier, France. Key EDEN milestones are followed up at SC level; per SP or HIT each respective leader (all members of SC) is responsible for milestones and deliverables. During this reporting neither gender nor ethical issues were raised by EDEN partners. A careful watch is being kept over progress.

2. Project meetings

Two steering-committee meetings (SCM) were organised respectively in Brno (January 2008) and Paris (September 2008). We tried this new frequency following suggestions from various origins, and attempts to remedy to the overloaded diaries of all the SCM participants. This came with the major disadvantage to overlaod the agenda of the Paris SCM. For the last year of the project, we propose to re-establish the frequency of three SCM a year. This will be a good opportunity to prepare thoroughly the international conference to be held at the end of the project (Montpellier, May 2010).

Immediatly before the Paris SCM, a joint meeting was organised with the Epizone project (FP6): Network of Excellence for Epizootic Disease Diagnosis and Control. The mutual activities were presented and proposals wer made to strengthen the collaboration between the two projects. Epizone was mainly interested by our modelling and integrative approaches. Calls for proposals may be lauched by EPIZONE for small collaborative projects between EDEN and EPIZONE teams.

3. Audits

- No financial audit was required for the coordination in 2008.
- The scientific aspects of EDEN were evaluated by the *European Policy Evaluation Consortium* (EPEC), on behalf of DG Research. A questionnaire was filled and the

coordinator was interviewed by an EPEC reviewer. So far, we did not get any feed back from this evaluation.

4. Training

- At the end of October 2008, we organized in Paris a 3-day workshop on R_0 and disease-spread mathematical modeling, in collaboration with the French National Institute for Agronomic Research (INRA) and French School of Public Health (EHESP). This workshop targeted PhD students, post docs, and scientists actually involved in mathematical modeling. It was split into keynote lectures, communications and posters (2 days) and a 1-day tutorial. The EDEN project was presented by the coordinator and two keynote lectures were given by EDEN HIT leaders (DJ. Rogers and H. Heesterbeek). Nienke Hartemink (HIT mathematical modelling) was one of the trainers involved in the tutorial. One hundred and twenty five people attended the meeting (fig. Coord-01), with 24% of EDEN-related participants. The majority of partcipants were French (reflecting the meeting co-organisation with INRA and EHESP). Ultraperipheral European regions were represented (La Réunion, France), as well as Africa (Ethiopia, Madgascar), Asia (Korea, Japan), and North America (USA, Canada). The target population of young scientist was reached: 44% of PhD students and 6% of postdocs. This was the result of the financial support brought by the EDEN coordination (> 10,000 €), allowing very low workshop fees (free for PhD students). Many posters and communications are available on the EDEN website (http://www.eden-fp6project.net/).
- The EDEN coordinator was invited to present EDEN methods and results during a training course organised in Athens (Greece), 18th June 2008, in the framework of the CIRCE project (FP6: impact of climatic changes in the Mediterranean basin).

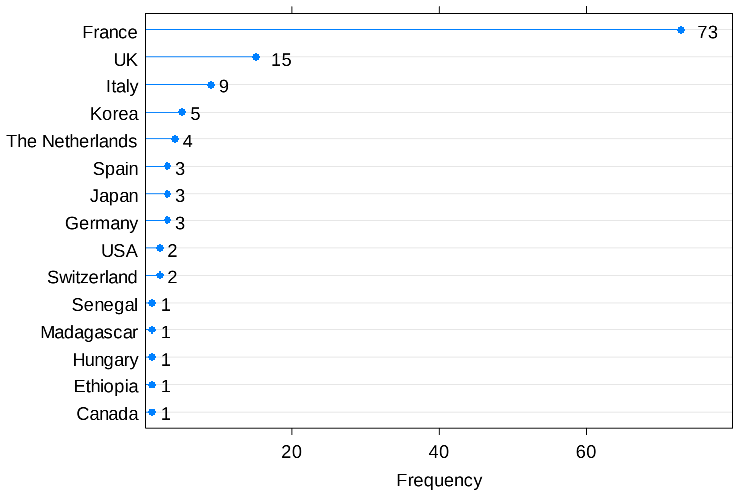


Fig. Coord-01. Participation to the R0 meeting organized in Paris, 28-30th October 2008.

- The EDEN coordinator organised a 2-week module in Montpellier (November 2008) on the surveillance of vector-borne diseases and vectors, in the framework of the Master of public health (Alfort vet school, CIRAD, Paris XI and XII universities). Several EDEN scientists gave lectures for this purpose (CIRAD, IRD, EID Méditerranée and Institut Pasteur), as well as the coordinator himself.
- The EDEN coordinator was invited by IZS Teramo (EDEN partner n°7, Italy) to contribute to the design and implementation of an on-line course on vectors and vector-borne diseases, funded by the Italian Ministry of Education, University and Research (28-30th

April 2008): *eMed, innovative e-learning system for the agricultural sector in the Mediterranean countries*.

4. Website

The master EDEN website (http://www.eden-fp6project.net/) was improved (graphical interface) and regularly updated. Through the website, we receive several requests every week from the general public, teachers, journalists… for information, leaflets, or interviews.

5. Newsletters

The publication of the EDEN newsletter was stopped after concertation with the EC scientific officer. The time saved was used to design and implement new versions of the EDEN leaflet and posters (July 2008) which were widely disseminated during the many scientific meetings attended by EDEN partners. The new leaflet was also directly sent to key stakeholders in the domain of health – environment, public health, and organisms involved in research, surveillance and control of vector populations and vector-borne diseases.

6. Scientific publications

Publications with an EDEN number

De La Rocque, S., Tran, A., Etter, E., Vial, L. & Hendrickx, G. 2007. Environmental changes, disease ecology and geographic information system-based tools for risk assessment. *Veterinaria Italiana*, 43: 381-391. EDEN0036.

De La Rocque, S., Rioux, J.A. & Slingenbergh, J. 2008. Climate change: effects on animal disease systems and implication for surveillance and control. *Revue scientifique et technique de l'Office international des Epizooties*, 27: 339-354. EDEN0088.

Proceedings

Lancelot, R., de La Rocque, S. & Chevalier, V. 2008. Bluetongue and Rift Valley fever in livestock: a climate change perspective with a special reference to Europe, the Middle East and Africa. In: Rowlinson, P., Steele, M. & Nefzaoui, A. (eds.) *Proceedings of the international conference on livestock and global climate change*. 2008 Cambridge University Press, 87-89.

Lancelot, R., Rogers, D. & Hendrickx, G. 2008. The EDEN consortium on emerging, vector-borne diseases: a simple recipe for a complex problem. In: ESOVE (ed.) *The 16th European Society for Vector Ecology Conference 2008*. Conference Programme & Abstract Book, p. 27.

7. International workshops / seminars / conferences

- European Society for Vector Ecology (Cambridge, UK, 25-26 March 2008)
- *International Conference on Livestock and Global Climate Change*, Hammamet (Tunisia), 17-19th May 2008
- *INRA-CIRAD International Colloquium*, Paris (OIE), 3rd June 2008: presentation of the EDEN integrative approach in front of an audience of directors of national and international agronomic research organisms and universities from > 50 countries worldwide.
- *Euro-Mediterranean scientific network meeting*, Paris, 25th June 2008: presentation of the EDEN integrative approach in front of an audience of directors of national and international research organisms and universities from the Mediterranean region.
- Organisation of an EDEN satellite meeting (2 * 3 hours) during the Xth European Multicolloquium or Parasitology (Paris, 27 Aug. 2008): participation of the coordinator,

the secretary and chairman of the steering committee, as well as several EDEN scientists from France, England, Belgium and the Netherlands.

- R_0 workshop in Paris (EDEN, INRA, EHESP, 28-30th October 2008): see above.

- Co-organisation with CIRDES (Centre International de Recherche-Développement sur l'élevage en zone sub-humide), CIRAD, IRD and Abomey University (Benin) of an international conference on "Demographic and climatic changes: impact on vector-borne diseases in West Africa". It was held in Ouidah (Benin) at the School of Public Health (24-26th Nov. 2008) and attended by ca. 150 scientists from northern, western and central Africa, and Europe (France, Belgium, The Netherlands, Germany), as well as the students of the *International Entomology Master* co-organised by IRD and Abomey university. The EDEN co-ordinator presented a keynote lecture on EDEN methods and results, and the possible implementation of a similar project in Africa. The conclusion of this communication is that densely-populated areas of Africa (Guinea gulf, Victoria lake basin, Ethiopian highlands…) are high-risk ecosystems for the emergence of vector-borne diseases. This idea was nicely summarised in a recent paper published by Jones et al. (2008) in *Nature* (Fig. Coord-02). Therefore, research and surveillance programmes should be implemented to assess, map and monitor the emergence risks according to environmental, social and economic factors. Indeed, many EDEN teams are already involved in somewhat sparse research programmes in Africa (malaria, trypanosomiasis, Lassa fever, plague, Crimean-Congo haemorragic fever, Rift Valley fever…). Nevertherless, there is a lack of global understanding and generic vision: we definetely miss an ambitious research programme on emerging, vector-borne diseases in Africa!

- Joint meeting of the French Veterinary and overseas academies (Paris, World Organisation of Animal Health, 28th Nov. 2008): presentation of EDEN results on the impact of climate change and other environmental factors on the emergence of vector-borne diseases.

- "France Vétérinaire International" meeting in Paris, Ministry of Agriculture, 4th Dec. 2008: presentation of possible implementation of a research programme on vector-borne diseases in Africa.

8. Other dissemination

- Many interviews in the scientific and general press, radio and TV. A record of these interventions is made by the CIRAD communication service and is available on request.

- Scientific committee of the exposition "Epidemik" at the Cité des Sciences de La Villette (Paris, October 2008 – May 2009). The work of the scientific committee and the script of the exposition were compiled in the edited book: Flahaut A., Zylberman P. (eds), 2008. *Des épidémies et des hommes*. Editions de la Martinière, 239 p."

- Participation to the "Fête de la Science": conference on emerging, vector-borne diseases in CIRADn Montpellier (> 100 people, general audience).

9. Networking

- Several formal and informal meetings in Brussels with DG Research, DG Dev and DG Sanco. Discussion on the utilization of EDEN results, and on the design of new FP7 projects.

- Joint meeting between the steering committees of EDEN and Epizone project: Paris, 24th Sept. 2008. Many informal discussions with the Epizone coordination (in particular, Johan Bongers). Development of bluetongue activities: modelling of spatial spread of the European BTV-8 epizootic, using EDEN methods and tools.

- Development of strong relationships with the *European Center for Disease Prevention and Control* (ECDC, Stockholm):
  - participation to several meetings and workshops, including Workshop on Linking Environmental and Infectious Disease Data, 28-29 May, 2008, Sigtuna. The EDEN contribution and recommendations were included in a joint EEA-JRC-WHO report "Impact of Europe's changing climate – 2008 indicator-based assessment" (EAA Report n°4/2008, JRC reference report n°JRC47756).
  - Involvment of many EDEN scientists in the ECDC expertise "Assement of magnitude and importance of vector-borne diseases in Europe". This project was carried out outside of EDEN activities, but the main ideas and results developed in the frame of EDEN were utilized and highlighted. An official report will be published by ECDC where EDEN will be acknowledged. Two special issues of the journal Eurosurveillance will be published with the results of the expertise, written by EDEN scientists.

Contractor contributions and changes in the consortium

Budget reallocation

- A survey was undertaken among EDEN partners to identify teams that had spent most of their budget (information available to the coordination) and that needed more to complete field work and / or data analysis and dissemination (including PhD thesis, postdocs, and participation to the annual meeting and the international conference). On this basis, proposals were made by the coordination to the steering committee (table Coord-01) and validated during the SC meeting in Paris, September 2008.

Partner n°	Short name	REALLOCATION PER PARTNER (Euros)
34	NHM	- 13 700
3	UCL	- 190 000
6	UNISAP	- 35 000
Total DONORS		**- 238 700**
7	IZS	35 000
2	FVM	75 000
9	CNM	15 000
11	IVB	10 000
14	HUESRI	15 000
19	DIDN	10 000
22	TAI	5 000
23	IMI	12 000
36	LSH&TM	29 700
37	SZIE	5 000
40	UoC	12 000
45	EBD	15 000
Total BENEFICIARIES		**238 700**

Table Coord-01. Reallocation of budget proposed to the steering committee.

- Transfer between UNISAP and IZS (€35,000) is explained below.
- Funds were taken from LSH&TM and given to NHM to pay a short-term contract for data management and preliminary analysis in the Leishmaniasis sub-project: this work should have been done by LHS&TM but was delayed because of illness of the team leader.
- The UCL partner (Louvain University, Belgium) had an unspent budget of €190,000, which was initially reserved for purchasing satellite images for EDEN partners. This budget was reallocated to other EDEN partners according to their requests.
- From a scientific-strategy viewpoint, the most important reallocation of budget was in favour of Utrech University, to allow the recruitement of a postdoc (Nienke Hartemink) to complete the development of mathematical models of disease transmission for the different sub-projects.

Consortium changes

- Partner n°18 (CEA, Italy) has transferred its rights and obligations to "Fondazione Edmund Mach (new partner n°50). Teams and activities remain unchanged.
- Partner n°6 (UNISAP Roma, Italy) has withdrawed from the project. Its activities and resources have been transferred to partner n°7 (IZS Teramo, Italy).

Request for no-cost extension

In agreement with reviewers' recommendations after the 2008 annual meeting in Brno, a 7-month no-cost extension was asked to the EC, to complete data analysis / modelling, and write publications combining the results over diseases and ecosystems, thus achieving the genericity and integration goals defined at the beginning of the project.
We also propose to hold an international conference in Montpellier, at the end of this no-cost extension period (May 2008), to present EDEN results to the international scientific community. The proceedings of this conference will be published as an edited book revisiting and extending the strategy document elaborated at the beginning of EDEN, and updated during the course of the project.

Project time table and status

The different project activities are reviewed for this reporting period (i.e. month 25-36-42). Deviations from Workplan are clarified on a case by case in section 2.

Sub-project tick-borne diseases: TBD WP1-5

TBD WP1 – Environment	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data collation and analysis																		
Finalize national data archives																		
National analyses and publications																		

TBD WP2 – Vectors	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data collation and analysis																		
Finalize national data archives																		
National analyses and publications																		
Field work																		
Final field samples where applicable																		

TBD WP3 - Public health	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data collation and analysis																		
Finalize national data archives																		
National analyses and publications																		

TBD WP4 - Reservoirs & animal hosts	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data collation and analysis																		
Finalize national data archives																		
National analyses and publications																		
Field work																		
Final samples where applicable																		

TBD WP5 – Data management	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Database development																		
Develop geo-ref multi-national spreadsheet																		
Enter information into network database																		
Integrated analyses and publications																		

Sub-project rodent-borne diseases: Robo WP1-5

Robo WP1 – Environment	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Climate and landscape structure																		
Detailed masting models																		
Local snow cover models																		
Local landscape change analysis																		
Envi-factors affecting host distribution patterns																		
Publications																		

Robo WP3 - Public health	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Public Health																		
Final hanta occurrence updates																		
Impact warm autumns on disease occurrence																		
Micro-array methodology development																		
Publications																		

Robo WP4 - Reservoirs and animal hosts	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Animal reservoirs and viruses																		
Rodent population dynamics and phylogeography																		
Sampling of rodents and their viruses																		
Rodent molecular work																		
Virus molecular work																		
Virus persistence lab experiments																		
Virus – host compatibility - experiments																		
Virus – host compatibility - molecular work																		
Publications																		

Robo WP5 - Data management	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Risk modelling and data management																		
Data standardisation																		
Information into network data base																		
Integrated risk models																		
Publications																		

Sub-project leishmaniasis: LEI WP1-5

LEI WP1 – Environment	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data acquisition and collation																		
Collect data																		
Collect field data																		
Publications																		

LEI WP2 – Vectors	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data acquisition and collation																		
Collect data																		
Collect field data																		
Construct data layers																		
Spatial analysis modelling																		
Molecular population analysis																		
Publications																		

LEI WP3 - Public health	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data acquisition and collation																		
Collect data																		
Construct data layers																		
Molecular identification of new isolates																		
Publications																		

LEI WP4 - Reservoirs and animal hosts	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data acquisition and collation																		
Collect data																		
Construct data layers																		
Spatial analysis modelling																		
Molecular identification of new isolates																		
Publications																		

LEI WP5 - Data management	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data acquisition and collation																		
Collate geo-referenced databases																		
Conduct integrated analyses																		
Publications																		

Sub-project West Nile virus: WNV WP1-5

WNV WP1 – Environment	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Co-ordinated data base standardization																		
Co-ordinated ecological mapping and GIS modelling																		
Data analysis and publications																		

WNV WP2 – Vectors	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Collection and identification of adult mosquitoes																		
Seasonal profile of ornithophilic and horse-biting mosquitoes																		
Collection and identification of bird ecto parasites/tes for virus																		
Overwintering of WNV in *Culex pipiens*																		
Data analysis and publications																		

WNV WP3 – Public Health	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Public health data bases																		
Data analysis and publications																		

WNV WP4 – Reservoir and animal hosts	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Bird trapping, blood-sampling																		
Serology Of WNV in migratory & resident birds																		
Serology of WNV in horses																		
Analysis and publications																		

WNV WP5 – Data management	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data analysis: epidemiology of WNV																		
Epidemiological modelling																		
Integrated publications																		

Sub-project malaria: MAL WP1-5

MAL WP1 - Environment	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Describe landscape in additional sites																		
Collect GPS referenced-field data																		
Construct spatial data layers																		
Spatial analysis and modelling																		
Publications																		

MAL WP2 – Vectors	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Collect field data on Anophelines																		
Process mosquitoes for Plasmodium																		
Study species morphology and genetics																		
Molecular population analysis																		
Study experimental vector competence																		
Construct spatial data layers																		
Spatial analysis and modelling																		
Publications																		

MAL WP3 - Public health	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data on imported and autochthonous cases																		
Study impact of human movement on malaria																		
Study impact of human activities on malaria																		
Study public health activities related to malaria																		
Construct spatial data layers																		
Spatial analysis and modelling																		
Publications																		

MAL WP5 - Data management	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Data acquisition and collation																		
Collate geo-referenced databases																		
Modelling analyses																		
Integrated papers																		

Sub-project Africa platform: AFR WP1-5

AFR WP1 - Environment	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Final field surveys																		
Final data analysis																		
Publications																		

AFR WP2a – RVF vector studies	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
TO BE COMPLETED																		
Final field surveys																		
Final data analysis																		
Publications																		

AFR WP2b – WNV vector studies	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
TO BE COMPLETED																		
Final field surveys																		
Final data analysis																		
Publications																		

AFR WP4 - Reservoir and animal hosts	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
DATA ACQUISITION AND COLLATION																		
Final field surveys																		
Final data analysis																		
Publications																		

AFR WP5 - Data management	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
DATABASE DEVELOPMENT																		
Data standardisation and harmonisation																		
Central data base																		
Integrated analysis																		
Integrated publications																		

Data management: WP6.1

	Normal EDEN duration												Proposed no-cost extension					
WP 6.1 - Data management HIT	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
EDEN DMT Website – IDEAS																		
Inquire for EDEN user needs at international gatherings																		
Update web based spatial data archive																		
Conduct web resource inventory																		
Compile data archive																		
Transform existing data sets																		
Develop new data sets																		
Develop EDEN PhD Web facility																		
Capacity building																		
Provide on-line help on DMT issues																		
Liaison with Sub-Projects																		
Develop distance learning tool																		

Information systems																		
Contribution to EDEN strategy document																		
Analysis of EDEN information system requirements																		
Development EDEN Information System																		
General																		
Annual report																		
EU Evaluation																		
EDEN SC meetings																		
EDEN International conference																		

Remote sensing: WP6.2

WP 6.2 - Remote sensing HIT	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
HRRS HIT																		
Validation of High resolution study sites																		
Establishment of conceptual models																		
Description of metadata																		
HR classifications																		
Diachronic analysis of land-use changes																		
Integrated activities with HITs, SPs																		
LRRS HIT																		
Low resolution image download																		
Low resolution image processing																		
Second generation statistical models developed																		
Second generation statistical models applied																		
Develop biological models if appropriate data available																		
Integrated activities with HITs, SPs																		

HIT PhD program: WP6.3

WP 6.3 - PhD program HIT	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
PhD Environmental change																		
PhD environmental change																		
Joint publications with SPs																		
PhD mathematical modelling																		
PhD mathematical modelling																		
Post Doc extension																		
Joint publications with SPs																		
PhD biodiversity																		
PhD biodiversity (thesis finalized)																		
Review submitted publications																		

Overall integration – tools and scenarios: WP7

WP 7 – Tools and scenarios HIT	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
EDEN Strategy Document																		
Meetings/Discussions Steering Committee																		
EDEN international conference																		
Final version SD = Conf. proceedings																		
Project annual meetings																		

Management, training and dissemination coordination: WP8

WP 8 – Coordination	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Permanent coordination tasks																		
Contact point with EU																		
Day to day co-ordination & troubleshooting																		
Administrative guidance for partners																		
Follow up of project milestones																		
Financial monitoring																		
Facilitate communication between project teams																		
Liaise for gender, personnel and ethical issues																		
Coordination of meetings and reporting																		
Steering committee meetings																		
Project review																		
External Audits (as applicable)																		
EDEN Annual Meetings (AGM)																		
Training coordination																		
Dissemination coordination																		
EDEN Website updates																		
Advisory Group (AG) meetings																		
EDEN Publication abstracts update																		
Implement dissemination plan																		

Section 4 – EDEN Dissemination plan

IS NOW INCLUDED IN EDEN STRATEGY DOCUMENT
HAS BEEN REMOVED FROM HERE TO AVOID DUPLICATION MISTAKES

2.2. Periodic management report

2.3. Periodic report on the distribution of the Community's contribution

2.4 – Draft planning for last 19 months (M49-M67)

Workpackages TBD1 to TBD5: Tick borne diseases

TBD personnel exchanges planned in the next reporting period include:
Dr Alexandru Vladimirescu and Miss Claudia Coipan (MIRDMI) will visit Dr Lise Gern's laboratory (Neuchâtel) in Dec. 2008.

WP TBD 1 – Landscape, biotopes and habitats

Work package number	TBD1	**Start date or starting event:**		49		
Participant id	ZOOX	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant	1	1	1	1	1	1	1
Participant id	IMI	NEIKER	OEK	PHA	CCDPC	NIRDMI	Total
Person-months / participant	1	1	1	1	1	1	13

Objectives

To complete the acquisition and collation of information on climate and landscape changes in relation to regional and local changes in the incidence of TBDs, specifically tick-borne encephalitis (TBE).

Work performed during previous reporting periods

A database of standardized climatic data from selected sites in each partner country over the period 1970-2004 has been built. Almost all partners have completed the acquisition of daily min and max temperature, rainfall, snow cover. Time-series analyses have been completed and published for the Baltic States. Exceptional weather during 2006-2007 was characterized in relation to the spike in TBE incidence in 2006 in several, but not all, countries. Land cover/land use change in the five selected (CEE) countries have been investigated and indicate a modest increase in the overall extent of forests and woodlands and a very marked abrupt decrease of agricultural activities and productivity in some Eastern European countries after the end of Communism. In a collaboration between EDEN-TBD and HIT-HRRS (partner UCL), a land cover map of NE Latvia has been developed based on Landsat imagery, and validated by field work. Land cover, land use and land tenure were all shown to be significant factors in determining differential probability of TBE cases in rural parishes in Latvia.

Description of work

Up-dates of environmental data to the end of 2008 will be completed. Partners will remain vigilant for the availability of additional data sources, especially in response to questions that arise during analyses and preparation of publications.

Deliverables

D TBD01 – Update to end 2008 of spatial database of standardized climatic data for each TBD partner country at multinational levels (M18-24-36-48-60).

Milestones and expected result

M TBD18 – The spatial database on climatic data and land cover/land use change are periodically updated. (M36-54)

WP TBD 2 – Vector bionomics and competence

Work package number	TBD2	Start date or starting event:			49			
Participant id	ZOO X	IVB	NIRDMI	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months/ participant	1	1	1	1	1	1	1	1
Participant id	IMI	OEK	NEIKER	PHA	CCDPC	VRMI		Total
Person-months/participant	1	1	1	1	1	4		17

Objectives

The general objective of this WP is to link tick population changes and dynamics with regional and local changes in the incidence of TBDs in the cluster of countries with resident EDEN-TBD teams. During the period of months 25-42 the specific objectives are:

- To complete the development and dissemination to each TBD partner institute state-of-the-art molecular tools and standardized protocols for (i) diagnosis of multiple infections in ticks and (ii) identification of tick blood meals.
- To analyze at the national and sub-national scale historical tick population changes in relation to regional and local changes in the incidence of TBDs.
- To undertake fieldwork to collect data on different patterns of tick seasonal population dynamics (processes) associated with different epidemiological patterns in each partner country.
- To analyse samples of field-collected ticks from each site for their infection prevalence and blood meal identity.

Work performed during previous reporting periods

The German team (LMU), together with the Slovenian (IMI) and Swiss teams, has developed molecular tools and standard protocols for the detection of pathogens in ticks, and is continuing to develop tools for the identification of tick blood meals. In addition to the 3 training workshops on Real Time PCR during period 1, exchanges between Munich and various teams have ensured adequate further training for those who need it. Processing of ticks has been started by most teams, as far as limited funds permit, and some preliminary data on infection prevalence have been achieved.

Each partner has searched for available data on changes in tick populations from 1970 to the present. This search will go on, as new sources are continually coming to light. Available data are very variable in terms of quality and quantity. Eighty-one field sites have being selected for monthly tick sampling over periods 2, 3 and 4. The sampling protocols have been defined and implemented. This is now complete. Ongoing analyses have revealed major increases in tick abundance in some areas, and highly variable patterns of tick seasonal dynamics from year to year and place to place, as expected. The planned numbers of nymphal and adult ticks have been collected from each site and stored (frozen) for analysis of their infection status and blood meal identity. Tick infection prevalence: ticks from 11 countries were screened for infection with TBE virus, *Borrelia burgdorferi* s.l. strains, *Anaplasma phagocytophilum* and *Babesia* spp., with the material from the remaining two countries partially processed. Procedures for blood meal identity in field-collected ticks are under way in at least two laboratories.

Description of work

Field data on the following from across Europe will be compared and related to environmental factors: a) tick seasonal dynamics, b) infection prevalence of four pathogens in field-collected ticks, and c) rodent abundance, infection status and tick infestations. Causes underlying the observed patterns will be sought.

Any remaining samples of ticks and rodent sera will be screened for infection, as the need arises following

analyses of the results to date, according to available funds permit. In Hungary in 2007, there were two epidemics of TBE (35 patients from two farms) caused by the consumption of raw goat milk. If the farmers agree, ticks (and rodents?) will be collected (VRMI) from the pastures where the goats were grazing, and screened by PCR for TBEV (VRMI and OEK).
Deliverables D TBD16 – Final measures of the geographically variable prevalence of infection with tick-borne pathogens in ticks (M54). D TBD17 – Information on tick blood meal identity in relation to infection prevalence (M54 Unlikely).
Milestones and expected result M TBD19 - The first data to indicate the successful transfer of the methods of blood meal analysis from Neuchâtel to LMU (M51).

WP TBD 3 – Public health and human activities

Work package number	TBD3	Start date or starting event:		49			
Participant id	NIRDMI	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant	1	1	1	1	1	1	1
Participant id	IMI	CNM	OEK	PHA	CCDPC	ZOOX	Total
Person-months / participant	1		1	1	1	1	12

Objectives To complete the acquisition and collation of information on public health activities and sociological factors relevant to human exposure to infected ticks, in relation to regional and local changes in the incidence of TBDs, specifically tick-borne encephalitis (TBE).
Work performed during previous reporting periods Records of TBDs (principally TBE) at local and regional scales over the period 1970-present were extracted from national Public Health agencies by most partners. Detailed data on such factors as the age and sex, and sociological markers of potential changes in human behaviour associated with agricultural practices, poverty and wealth, have been proved relevant to an increased risk of exposure to ticks. Additional data on seasonal patterns at regional scales were collected. Interpretation benefited from detailed analysis of the spike in TBE incidence that occurred in 2006 in some, but not all, countries. In addition, almost all partners have also recorded relevant public health information, including an analysis of the impact of improved methods of diagnosis on official national records or vaccination coverage. From the growing bank of data that has been accumulated, an increasingly clear picture has emerged of the changes in public health activities (principally vaccination) and sociological factors related to contact between humans and infected ticks in forests over the past 35 years. **Description of work** Up-dates of epidemiological data to the end of 2008 will be completed. Detailed analyses of the demographic patterns of TBE incidence will strengthen our interpretations of variable risk factors in time and space.
Deliverables D TBD06 – Standardized database, **updated to end 2008**, of national records of historical trends in local and regional incidence of TBDs (M60).

Milestones and expected result
M TBD15 - Spatial patterns in sociological factors and land cover/land use (WP1) in relation to epidemiological data will direct the further selection of particular indices of sociological and behavioural change that may have increased human contact with infected ticks (M48).

WP TBD 4 – Animal reservoirs

Work package number	TBD4	**Start date or starting event:**			49		
Participant id	NIRDMI	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant							
Participant id	IMI	NEIKER	OEK	PHA	CCDPC	ZOOX	Total
Person-months / participant				1		1	2

Objectives

The general objective of this WP is to describe the impact of environmental changes or human intervention on host availability and the abundance and/or seasonal dynamics of local tick populations. During months 25-42 the specific objective will be:

- To map regional and local changes in the distribution and abundance of wildlife and livestock vertebrate hosts for ticks.
- To continue to collect field data to establish the quantitative relationship between variable ungulate host availability on tick infestation patterns on rodents and infection prevalence in ticks and rodents.

Work performed during previous reporting periods

A large amount of valuable data on large animal hosts for ticks, both domestic livestock (cattle, sheep, goats) and wildlife species (especially deer and wild boar, but also mouflon and wolves), was acquired and collated. These data have been compiled into standardized databases prior to presenting the information in mapped formats. The importance of the different types of livestock for maintaining tick populations varies from country to country depending on husbandry practices, such as the extent of outdoor grazing and seasonal patterns of grazing at different altitude. In addition, protocols and grids for rodent trapping were established at 3 sites in Italy and at 2 sites in Slovakia. Seasonal variations in the rodent populations and infestation levels of *I. ricinus* larvae and nymphs have been monitored over 2 years in Italy and 3 years in Slovakia, and ticks were collected from these rodents. Diagnosis of infection prevalence with tick-borne pathogens at each site has proceeded. These intensive field studies complement the GIS spatial analyses carried out in Italy, helping to inform us about the possible consequences of changing deer abundance on the transmission potential on various tick-borne pathogens. Rodent trapping was carried out in Latvia in collaboration with EDEN-ROBO to explore the colonization of abandoned agricultural land by the transmission hosts of TBE virus and rodent-borne viruses.

Description of work

Preserved rodents trapped in Latvia will be screened for TBE virus infection prevalence and the results will be analysed with respect to habitat type (forest, bush, fallow, field, the first two associated with the abandonment of agricultural land).

Deliverables

D TBD19 – Database with ROBO and HRRS on rodent populations and viral infection prevalence related to habitat changes in Latvia (M60).

D TBD22 – Indices of deer (and wild boar) abundance at each site where ticks are sampled (M52 Unlikely).
Milestones and expected result M TBD 16 - Consolidated results of serological evidence of infection in rodents with TBE virus (M42). M TBD 20 - Trial indices of deer abundance at selected sites (M42).

WP TBD 5 – Data management and cross disciplinary modeling

Work package number	TBD4	**Start date or starting event:**			49		
Participant id	NIRDMI	IVB	CEA-FEM	DIDN	LMU	SAS	TAI
Person-months / participant	1	1	1	1	1	1	1

Participant id	IMI	OEK	NEIKER	VRMI	PHA	CCDPC	ZOOX	Total
Person-months / participant	1	1	1	1	1	1	8	21

Objectives

The general objective of this WP is to use data generated by WP1-2-3-4 to develop epidemiological models of TBD risk to predict changes in TBD systems driven by landscape and climate changes as well as socio-economical changes. During months 13-30 the specific objectives are:

- To develop GIS databases of all information acquired from WP1-4.
- To develop a model of the predictor variables of the observed epidemiological changes by seeking correlations between patterns of disease and patterns of environmental and sociological factors.

Work performed during previous reporting periods

Over the first periods, a huge array of data sets acquired by each partner was sent to be collated by partner ZOOX. Many parts of the geo-referenced multi-national databases have been posted on the EDEN web site. Records of the annual incidence of TBE in each administration district in each partner country from 1970-2005 (WP3), were compiled into a common database and presented in both digital and mapped form. Climatic data have also been imported. Databases for information on other factors (land cover [WP1], vectors [WP2], sociological changes [WP3] and hosts [WP4]) are as complete as is possible given the availability of information. The abundance of the biological and sociological data, together with their non-uniformity, make the compilation of a single database much more complex and time demanding than was first imagined. The timing of statistically significant increases in the incidence of TBE varied between and within countries. No one factor shows consistent correlations with the spatio-temporal patterns of epidemiological change, as most factors showed considerable consistency within any one country. A working hypothesis was developed that emphasizes the synergy between independent, but indirectly linked, socio-economic and human-induced environmental changes in determining the spatio-temporal heterogeneity in TBE upsurges. As more data on environmental and socio-economic variables have been acquired and collated into databases, mostly at national levels but sometimes at county level, the increasing body of evidence has been substantiated, and in no way yet falsified, this analytical framework. Explanations for the upsurge of tick-borne diseases in CEE countries must now recognize not only an increase in risk though more infected ticks, but also an increase in human exposure to that risk through changing behaviour. This was emphasized by an analysis of environmental, entomological and sociological explanations for the spike in TBE incidence in 2006 in several, but not all, countries. It was apparently due more to human activities than to tick population dynamics in response to weather. Environmental conditions in northern Italy have been analysed and presented spatially. TBE risk mapping at national scales has started in collaboration with HIT-LRRS; the shifting distribution of TBE cases from 1970 to 2004 in Hungary is being analysed to search for environmental correlates.

Description of work Each partner will prepare publications based on the analysis of their own historical and new field and laboratory data. Comparative analyses of data from multiple partners will continue and be undertaken even more actively. To this end, all remaining data will be collated and posted on the EDEN-TBD web site. A more sophisticated common multi-national geo-referenced database of epidemiological, environmental, biological and sociological information, 1970-present, will be developed in collaboration with HIT-DMT. The development of an agent-based model in collaboration with HIT-HRRS will aim to predict geo-specific risk based on human socio-economic and environmental factors. This will incorporate individual human decisions concerning activities that might incur exposure to infected ticks on the basis of economic status, leisure pursuits, vaccination protection, risk awareness and the differential probability of infected ticks in forest habitats. All partners will consider and actively explore national and international opportunities to build on the significant advances made within EDEN, and to implement our new and deeper understanding of the underlying causes of the dynamics of tick-borne disease systems for public health benefits.
Deliverables D TBD 13 - An **up-dated** common multi-national geo-referenced database of epidemiological, environmental, biological and sociological information, 1970-present (M60). D TBD 20 – Time series and multi-variate regression analyses to establish correlations, and possible causality, between the epidemiological, environmental and sociological data from WP1-4 (M36-48). D TBD 25 - TBE risk maps for various countries (M67).
Milestones and expected result M TBD 11 - Definition of structure and components of database appropriate for the nature of the information (quantitative, qualitative, discrete and continuous) (M52).

Workpackages ROBO1 to ROBO5: Rodent borne diseases

ROBO personnel exchanges planned in the next reporting period include:

WP ROBO 1 – Landscapes, biotopes and habitats

Work package number	Robo1	Start date or starting event:					49
Participant id	METLA	UA	CEA				Total
Person-months / participant	2	3	1				6

Objectives

The overall objective of this WP is to understand patterns and processes linked to climate and the environment which (may) have an impact on, and will be related to the regional rodent/virus diversity and population dynamics in Europe.

Work performed during previous reporting periods

A list of working hypotheses was drawn of the climate and environmental parameters to be included in the regional spatial databases and models. Data compilation in progress on masting, forest resources and snow cover (Metla, UA, CEA) with assistance from the HIT data management team (EuroAegis). High resolution land-use and land-cover satellite imagery for each field site is in process with assistance from the HIT high resolution remote sensing (UCL). Data compilation for two main study regions, Belgium and Finland for a regional model on forest fragmentation patterns is proceeding.

The Finnish and Slovenian human data set sent to LR HIT. Belgian results on environmental correlated to PUUV published. Swedish LR work on human data goes on. UK cowpox work with environmental correlates started. The compilation of pan-European environmental data continues.

The masting dynamics affecting rodents and consequently human hanta epidemiology analysed. The first model on climatic variables affecting masting completed. Italian masting monitoring continues.

Italian snow data data sets.

HR and LR analyses of host and virus occurrence running for field sites and national data sets

Description of work

More detailed models on masting
HR and LR analyses completed
Pan European and local maps for factors affecting the potential host distributions (with HITs)

Local snow cover models

Deliverables

D Robo-01: Pan-European regional (1-8km raster data and vector data) and local spatial data base on masting, forest resources and snow cover (M60).

D Robo-03: Local models on masting dynamics (M67).

D Robo-04: models on forest fragmentation patterns (M67).

D Robo-05: Regional model on snow cover trends (M48 Unlikely).

D Robo-06: Ground-supervised local high resolution landscape change models for selected study sites where relevant changes have occurred, (Latvia) (M67).

Milestones and expected result

M Robo03 - Spatial data layers are used to generate spatial analysis models for remaining countries(M36).

M Robo04 - Model outputs are available and allow for (i) multi-factorial spatial analysis on virus/rodent

dynamics and (ii) extrapolation to other parts of Europe at later stage of project (WP5) (M60). M ROBO 11 - Environmental data used for understanding the distribution of roboviruses and their hosts as well as their dynamics and epidemiology (M60)

WP ROBO 2 – Vector bionomics and competence

Not relevant

WP ROBO 3 – Public health and human activities

Work package number	Robo3	Start date or starting event:			49		
Participant id	CEA	IMI	CBGP	SMI	UH.HI	ULIV	Total
Person-months / participant		2		10	8		20

Objectives The general objective of this WP is to describe and analyse the emergence patterns of diseases caused by hanta- and other roboviruses in Europe and adjacent regions.
Work performed during previous reporting periods Standardised human diagnostics protocols have been discussed in detail at KOM and subsequently, and are operational in all main human labs (UH.HI, SMI, IMI, and UA). In addition, student and sample exchanges between labs have been conducted. New microarrays being developed in parallel at UH-HI, (though not a deliverable but important for future sample processing in greater quantities). The existing data and general pattern of hantaviruses diseases known but there are still white spots especially in E Europe. Excellent data from some countries like Finland, Slovenia, Belgium, Sweden. New large materials from Italy and Switzerland have been analysed at SMI. Human disease data by LCMV complex is fragmentary, some data from Finland, Croatia, N Italy, UK exists, and they seem to show that in some regions at least in S Europe LCMV antibodies are more common in humans than hanta antibodies. Generally LCMV seropositivity is around 3-5% which can be considered a surprising result. A careful risk study by UH.HI showed that important risk factors w ere being a male, farmer and smoker. EDEN partners have screened materials from Italy, Switzerland, Hungary, and Croatian and the Slovenian forestry workers. The available historical European human data on hantavirus diseases, caused by PUUV DOBV and SAAV, have been compiled and will be published soon A review on the distribution and dynamics of hanta viral diseases in Europe was published. Diagnostics and methods have been updated and published, including the developing in microarrays methodology The role of human to human PUUV transmission excluded New human materials screened **Description of work** Latest updates on hanta disease occurrence More analyses on the impact of warm autumns on the seasonality of hanta disease Further developments in microarray methodology
Deliverables D Robo-07-b. Standardised human diagnostics microarrays ready for human diagnostic (M54).

D Robo-09. Updates of the second generation publications of observed human rodent-borne virus presence and serological prevalence in Europe (M36). D-9-b. Updates of the third generation data on human ROBO presence.
Milestones and expected result M ROBO06 - Updated maps of observed human roboviral presence in Europe (M 55) M ROBO07 – Serological data, especially those from groups at highest risk, contribute to further target work in WP 4 (M36-54)

WP ROBO 4 – Animal reservoirs

Work package number	Robo4	Start date or starting event:	49

Participant id	METLA	UH.HI	SMI	ULIV	UA	CBGP	CEA
Person-months / participant	20	22	14	12	7	15	5
Participant id	IMI						Total
Person-months / participant	4						99

Objectives

The general objective of this WP is to understand and quantify the role of the rodent reservoir in the distribution and transmission of identified emerging robo diseases. The specific objectives during the next 18 months are:

- To further train local teams for rodentological and virological sampling for field/disease surveillance and monitoring; at this stage this concerns candidate countries and adjacent regions particularly.
- To improve understanding and knowledge of the distribution of rodent reservoir species and the robovirus species they harbour in Europe, and establish their phylogeography and the importance of phylogeographic contact zones.
- To further reinforce reasonable network of local study sites for sampling for various purposes and representative monitoring.
- To test how environmental factors affect the virus persistence in different environmental conditions outside the host, which is critical for modelling and control of human infections.
- To understand the robo virus transmisson and density-dependence of robo dynamics in the host populations
- To understand the virus shedding dynamics and kinetics in the host during the infection
- To understand factors affecting temporal MHC-variation in hosts in relation robo dynamics, and to understand the dynamics of genetic variation and reassortment also at the local scale

Work performed during previous reporting periods

The presence or absence of hantaviral diseases is now rather well-known for many areas (but not all). Presence of arenaviruses (LCMV "complex"), cowpox and Borna is more fragmentally known. On the other hand, seasonal and long-term hantaviral dynamics are reasonably well-known only for selected countries, but for the rest of robos, dynamical aspects are so far poorly known. While the basic patterns of hanta dynamics are known (masting induced outbreaks in temperate zone and predation driven vole cycles in boreal forests), the contrast holds for arenas.

Various go and hit, annual/biannual and intensive trapping programs continued. Kinetics and shedding of PUUV were studies. The cyt-b and MhC phylogeography of the bank vole were analysed. New PUUV lineages were analysed. Details of transmission dynamics of PUUV both in cyclic populations in the north and mast driven populations in Belgium were studied, and modelling started. Cowpox dynamics and environmental correlated studies in the UK. The role of MHC in hantavirus risks for rodents were analysed, and the role of kinship in hantavirus dispersal was found.

Monitorings were continued, and new study on the impact of habitat change on robo and TBE dynamics was started in Latvia. Intensive new studies indicate interesting differences in immunological genetics in the bank vole in respect of PUUV. First long term data on LCMV in *Apodemus* mice submitted. Not a breakthrough yet on LCMV complex strain variation and identity. Further studies were done on the shedding kinetics of PUUV by bankvoles in nature. Detailed studies completed and being completed on PUUV dynamics in bank vole dynamics in Belgium and Finland.

Description of work

Long term intensive monitorings in Finland, Belgium and Italy will be completed and data sets analysed for PUUV transmission dynamics in relation to cyclicity, seasonality, changes in rodent population structure etc. This will be a basis for modelling work.
The analyses of material with long delays will be completed (e.g. Poland and Croatia)
The virus shedding patterns in nature by wild bank voles analysed in detail.
The Latvian Robo – TBE landscape data analyses will be completed. The Finnish forestry impact study will be completed.
The LCMV sequencing will finally succeed, and several new zoonotic LCMV strains will be recovered.
Detailed immunogenetic studies will go on. New analyses will be done on the relation of various genetic markers, PUUV phylogeography and bank vole susceptibility in Europe.
The detailed analyses on reassortment genetics continue, and the frequency and survival of reassortments analysed. Special attention will be paid to the newly found PUUV contact zone in NW Finland.

The analyses of cowpox landscape dynamics in UK and cowpox sequencing studies will be completed.

Deliverables

D Robo-10. Further priming of highly sensitive real-time PCRs and completing the rodent-borne virus microarrays (M60).

D Robo-13. Further publications on rodent and virus distributions, genetics and phylogeographies, transmission dynamics, virus-host competence, and viral kinetics during the infection (a lot achieved, but will of course go on M67).

D Robo-14. GIS analysis of spatial data sets collated at both high resolution longitudinal study sites (M67).

D Robo-18. The first results on effect of forestation and fallow of previous agricultural land Latvia on the habitat selection and distribution of ROBO- and TBE- carrying rodents (M60).

D Robo-19. EDEN Robo documents on field sampling and animal ethics in wild rodent research (M60).

D Robo-20. Result of the shedding patterns of PUUV in nature (M60)

D ROBO-21. Detailed results on the rodent-borne virus and host genetic variation in the course of strong rodent host fluctuations (M60).

Milestones and expected result

M ROBO09 - Publications on all presented topics and second generation maps of observed rodent and rodent-borne virus reservoir presence in Europe (M60).

New data on the occurrence of rodent-borne diseases from poorly know countries like Bosnia, Poland, Latvia. Further data on MHC analyses in relation to PUUV infectivity and on MHC phylogeography of bank voles. Completion of *Apodemus agrarius* phylogeography in relation to the distribution of Saaremaa (western palearctic) and Hantaan (eastern paleartcic) viruses. Detailed analyses on density dependence and seasonality of PUUV and DOBV: from the long term monitorings in Finland and Italy. First data on host-virus compatibility tests in Denmark. ROBO sampling document completed.

WP ROBO 5 – Data management and cross disciplinary modeling

Work package number	Robo5	Start date or starting event:				49	
Participant id	METLA	CBGP	UA	ULIV	CEA		Total
Person-months / participant	2	5	12	2	2		23

Objectives

The objective of this WP is to model and predict, using data generated by WP1-3-4, spatial and temporal transmission patterns for all major emerging hanta- and other roboviruses in Europe using an integrated health/environment approach.

Work performed during previous reporting periods

A published model for hantavirus (Sauvage et al. 2004) has been simplified and used to find a preliminary equation for R0. The most interesting feature of Sauvage's model after "dissection" is that the virus is more sensitive to parameters determining abundance of rodents than it is to survival of the virus outside the host. Still, indirect transmission is necessary to explain the observed seroprevalences but abundance (and dynamics) of hosts is more important for determining persistence of the virus (and whether there is a real risk to humans).

Environmental parameters were related to all trapping grids in Belgium and a risk model was made on the PUUV prevalence and density of bank voles. This work was done in cooperation with the high-resolution HIT team. Finnish transmission data have been started to be modelled. The landscape variables in relation to rodent-virus spatial patterns at the Finnish intensive site started. LR analyses of Finnish human data started. The LR landscape patterns of rodent-borne viruses in the French intensive site completed.

Using the human and rodent data from Central Finland, the seasonal and cyclic dynamics of human NE was modelled. The HR HIT analyses for Belgium and Finland continued. Ecological niche modeling continued in UA. Mathematical modelling of Finnish and Belgium data sets getting momentum. The Swedish LR will develop further.

Description of work

The mathematical modeling will be completed with HIT and Lyon groups, both for Finland and Belgium.
Ecological niche modeling completed (UA).
Finnish large human data set LR analysed (LR-HIT)
Updates on the Swedish human LR work completed (LR-HIT)
Slovenian LR work completed (LR-HIT)
Finnish and Belgian HR-HIT work will be completed.

Joint multipathogen risk maps

Deliverables

D Robo-15. Exploratory area-wide GIS model for the testing of ROBO risk assessment approaches (M67).

D Robo-16. Exploratory local high resolution GIS model for each field site for the testing of spatial epidemiology hypothesis and health-environment relationships (M67).

Milestones and expected result

M ROBO10 - Relevant spatial data are made available to include in GIS model (M 54)

Workpackages LEI1 to LEI5: Leishmaniasis

LEI personnel exchanges planned in the next reporting period include:

Dr Jonathan Cox with research assistant (partner LSHTM 36) and Dr Paul Ready (co-ordinator NHM 34) to ISS 12 and UB 40 to provide GIS and statistics training, if local skills in place.

Dr Yusuf Ozbel with geographer (partner EUMS 35) to LSHTM for GIS and statistics training.

WP LEI 1 – Landscapes, biotopes and habitats

Work package number	LEI WP1	Start date or starting event:		49			
Participant id	ISS	NHM	**LSHTM**	UB	UM1	CNM	IHMT
Person-months /participant	1	1	0.5	1	0.5	1	0
Participant id	EUMS	SZIE	UoC				Total
Person-months /participant	1	0.5	0.5				7

Objectives

The general objective is to quantify the relative roles of specific environmental and climatic factors as determinants of leishmaniasis foci.

The specific objective will be to match the results with vector/ disease data from other WPs, to identify indicators of foci within the 3 initial study regions [in Catalonian Spain (Andorra-Lleida-Barcelona; UB, NHM, LSH&TM), neighbouring France (Andorra-Toulouse-Carcassonne; UM1, NHM, LSH&TM) and northwest Italy (Torino-Genova-France; ISS)] and to test predictions of foci in the countries of all partners.

Work performed during previous reporting periods

Field work was started in summer 2005 in each of the three designated regions: Catalonian Spain (Andorra-Lleida-Barcelona), neighbouring France (Andorra-Toulouse-Carcassonne) and northwest Italy (Torino-Genova-France). In a first step, southern France was selected as a model focus, where environmental descriptors were included in a Palm database tested in the field. RS data and ground records for climate and land cover were obtained for the same region. In 2006 and 2007, such work was carried out in the Catalonian and Italian regions, for which RS data and ground records for climate and land cover were matched with new sandfly records in a preliminary analysis. Based on the early progress, partners in Madrid, Granada (part of UB 38), Turkey and Hungary all decided to carry out surveys in WP2 and WP4 from 2006, with a requirement for ground records and RS data for climate and land cover. Greece (UoC 40) continued with a pre-EDEN spatial mapping project (WP2, WP3, WP4) in the Attica region.

In 2007, the plan was to focus on analysis, but this was changed because of 1) the need to obtain more complete data sets for WP2 and WP4 from all regions, and 2) the illness of the PI of LSHTM 36. Nevertheless, a training workshop for the manipulation of data layers in ArcView was held in Barcelona (month 30), high resolution analysis of data from southwest France was carried out in Leuvan in collaboration between NHM 34 and the Environmental Change HIT (month 30), and priorities for collecting new field data were agreed with all partners in a workshop in Piacenza, Italy (month 31) before the transmission season in summer 2007. RS datalayers were collected for the Attica focus in Greece, but not for Hungary because of limited distribution of vectors and absence of leishmaniasis, and not for Turkey because of the need to establish altitudinal transects with sufficiently high densities of sandflies and prevalences of leishmaniasis.

In 2008, the long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated. It was only in September 2008 that approval to recruit a research assistant to help LSHTM 36 was granted and a suitable assistant identified. A workshop was held in Granada, Spain (month 44), attended by all partners except UM1, from which it was clear that partners CNM 9, ISS 12 and UB 38 did not have adequate support from local geographers.

Description of work

In M49-60, the focus will be on data processing and analysis. This will be co-ordinated by NHM (with one PhD) and LSHTM (with one part-time post-doctoral geographer and one part-time research assistant). There are now plans to assist the teams without geographers (see 1.4.1.3. above; CNM 9 was assisted by a visit from LSHTM 36 and NHM 34 in December 2008). Field collection of environmental data will be completed by partners.

Deliverables

Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project.

D LEI13 – Standardized spatial GIS data base of environmental and climate descriptors for all study regions. All outstanding raw data sets will be obtained and processed (M54).

D LEI14 – Standardized descriptions and maps (GIS models) of environments and climates in all study regions (M54).

Milestones and expected results

M LEI05 - Preliminary spatial model outputs are available and allow for 1) spatial analysis of vector/ disease/ environment associations in first 3 study regions (WP5; M18), and 2) extrapolation to other regions (M60).

M LEI32 – Timetable of visits for data analysis and collaborative preparation of publications (M53): to be agreed after EDEN AGM 2009 (M51) and WorldLeish4 congress in India (M52).

WP LEI 2 – Vector bionomics and competence

Work package number	LEI WP2	Start date or starting event:				49
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	2	3	0	8.25	5	1	7
Participant id	UB	UM1	UoC				Total
Person-months / participant	5	0	5				36.25

Objectives

The general objective of this WP is to explore the variations and recent changes in the abundance of vector species and races in contrasted ecotopes.

The specific objectives are: to analyse the geo-referenced past records of the sandfly vectors of leishmaniasis; for the more experienced teams to acquire field data from their study regions in Catalonian Spain (UB 38), neighbouring France (NHM 34, LSH&TM 36, UM1 39) and northwest Italy (ISS 12), and the results analysed in relation to environmental and disease data from other WPs to identify indicators of foci; and, to investigate population structure and dispersal in relation to environmental variations using molecular characterization of some sandfly species in some regions.

Work performed during previous reporting periods

An historical database on absence/presence and density data for each sandfly species, within foci and non-foci, based on past records was developed by all partners and completed by most partners in previous years. By M48, only the historical databases for northern and southern Spain (UB 40) and the updated historical database for Greece (UoC 40) had not been received by the co-ordinator (NHM 34).

New collections in 2005-2008 specifically targeted the range of climate and environmental zones in each of the study regions, starting with Catalonian Spain, France and Italy, then Spain (Granada, Madrid), Greece (Athens), Hungary (southwest) and Turkey (Centre-South). In 2008, field surveys of sandflies were continued by partners CNM 9, ISS 12, NHM 34, EUMS 35, SZIE 37 and UB 38. However, surveys carried out in Hungary were not standardized, and this should be rectified in the last period of the project.

Data processing and analysis was carried out for the data from the model region of southwest France (NHM 34, LSHTM 36), with the priority for the other regions being changed from analysis to the collection of new data to obtain more complete data sets. Training for other teams was provided in two workshops, attended by all the designated partners. A workshop in Barcelona (M30) focused on further training of the Spanish teams to use ArcView software with a statistical package (STATA) for spatial analyses. In a workshop in Piacenza (M31), the focus was on planning sandfly (and canine leishmaniasis) surveys. A workshop was held in Granada, Spain (month 44), attended by all partners except UM1, from which it was clear that partners CNM 9, ISS 12 and UB 38 did not have adequate support from local geographers. There are now plans to assist these teams (see 1.4.1.3. above; CNM 9 was assisted by a visit from LSHTM 36 and NHM 34 in December 2008).

Population structure and dispersal in relation to environmental variations were investigated using molecular characterization of some sandfly species in some regions. Populations of sandflies from a range of habitat fragments in southwest France were characterized at mitochondrial and nuclear loci, to investigate the effect of habitat fragmentation on the dispersal of sandfly vectors of leishmaniasis (NHM 34).

In 2008, the emphasis was to have been on data processing and analysis. However, the long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated. It was only September 2008 that approval to recruit a research assistant to help LSHTM 36 was granted and a suitable assistant identified.

Description of work

In M49-60, the focus will be on data processing and analysis. This will be co-ordinated by NHM (with one PhD) and LSHTM (with one part-time post-doctoral geographer and one part-time research assistant). There are now plans to assist the teams without geographers (see 1.4.1.3. above; CNM 9 was assisted by a visit from LSHTM 36 and NHM 34 in December 2008). Field collection of environmental data will be completed by partners ISS 12, NHM 34 and UB 38.

The need for new surveys to complete datasets will be assessed at EDEN AGM 2009 (M51), but will not be encouraged except in Hungary where vectors were unknown before EDEN.

Deliverables

Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project.

D LEI15 – Standardized spatial data base of EDEN records of sandfly species in each study region will be integrated for inter-regional analyses, with a new deadline to be agreed at EDEN AGM 2009 (M60).

Milestones and expected result

M LEI08 – Plans to be agreed for using data layers to generate spatial analysis models for western Europe and all Europe, when more complete datasets are available. A new deadline will be agreed at EDEN AGM 2009 (M51).

M LEI32 – Timetable of visits for data analysis and collaborative preparation of publications (M53): to be agreed after EDEN AGM 2009 (M51) and WorldLeish4 congress in India (M52).

WP LEI 3 – Public health and human activities

Work package number	LEI WP3	Start date or starting event:			49	
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	1	1	0	0	1	0	0
Participant id	UB	UM1	UoC				Total
Person-months / participant	1	0.5	1				5.5

Objectives

The general objective is to investigate recent spatial and temporal changes in human disease prevalence.

This will also be a specific objective, now that records of past and new cases of human leishmaniasis have been standardized. A report will be prepared on how to improve both case detection, by encouraging the use of standardized molecular tools, and case reporting, by proposing a standardized surveillance questionnaire for all countries.

Work performed during previous reporting periods

From national records, databases were prepared on the spatial and temporal distributions of prevalence rates of all past and new autochthonous cases caused by *Leishmania infantum* or *Le. tropica* strains in each partner's study region. The descriptive analysis of spatial and temporal changes of prevalence rates in all 9 regions in the past 20 years was started by partners UM1 and LSHTM 36.

In 2006-8, separate reports were prepared or updated with new cases for central Spain (CNM 9), south and east Spain (UB 38), France (UM1) and Italy (ISS 12), in preparation for a review of the epidemiological situation in southwest Europe. In each year, molecular characterization of *Leishmania* species and strains isolated from patients (classed by sex and age etc) was on-going.

At each EDEN AGM and in annual workshops, partners briefly discussed how to improve both case detection, by encouraging the use of standardized molecular tools, and case reporting, by proposing a standardized surveillance questionnaire for all countries. The discussion was led by partners associated with the national reference centres for France (UM1), Italy (ISS) and Spain (CNM), but there were not enough resources in 2006-8 to produce deliverables.

There was little activity in this workpackage in the fourth period, because of other priorities and too few partners attending the EMOP X meeting in Paris, August 2008. This will be remedied in the next period. The descriptive analysis of spatial & temporal changes of prevalence rates in the past 20 years was previously completed separately for southwest European countries, and one objective will be to publish this, to encourage extending the analysis to all regions.

Description of work

A report will be completed on how to standardize national surveillance systems and networks.

Finally, there will be a statistical analysis of spatial & temporal changes of prevalence rates in the past 20 years.

Deliverables

Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project.

D LEI07 – Descriptive analysis of spatial & temporal changes of prevalence rates in all 9 regions in the past 20 years, after a new deadline has been agreed at EDEN AGM 2009 (M67).

D LEI08 – Report on how to standardize national surveillance systems and networks, after a new deadline has been agreed at the WorldLeish4 Congress in India (M67), which will be attended by key partners: UM1 39, ISS 12, NHM 34 and LeishRisk project.

D LEI16 – Analysis of spatial & temporal changes of prevalence rates in some regions in the past 20 years, after a new deadline has been agreed at EDEN AGM 2009 (M60).
Milestones and expected result M LEI28 - Progress on completing the 3 deliverables will be monitored by the SP co-ordinator at the Steering Committee meeting in May 2009 (M55).

WP LEI 4 – Animal reservoirs

Work package number	LEI WP4		Start date or starting event:			49
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	0.5	4	0	0.5	2	8.5	4.5
Participant id	UB	UM1	UoC				Total
Person-months / participant	5	8	1				34

Objectives

The general objective is to quantify the role of dogs (as reservoirs) in the dynamics of the disease.

The specific objective is to use the geo-referenced databases to analyse spatial and temporal changes in disease prevalence and/or incidence in relation to climate and environmental descriptors in all study regions (see WP1).

For later stages of the project, case detection and reporting need to be improved, respectively, by encouraging the use of standardized molecular tools and a standardized surveillance questionnaire in all countries.

Work performed during previous reporting periods

Previously, regional databases were prepared from national, veterinarians' and partners' databases and merged in London (LSHTM 36), from which spatial and temporal distributions of prevalence and incidence rates were plotted for all past and new autochthonous cases caused by *Le. infantum* strains in some regions. Prospective serological surveys were carried out by many of the teams. Isolates were identified according to guidelines defined by the sub-project.

In 2006-7, the prevalence records from Spain, Portugal and France were mapped and used to produce the first risk map for the whole of Europe, led by LSHTM 36. Additional prospective surveys based on questionnaires posted to veterinarian clinics were carried out by partners CMN 9 (Spain), UM1 (France), ISS 12 (Italy), EUMS 35 (Turkey) and SZIE 37 (Hungary). Isolates provided new geographical records for the sub-Alpine region of Italy (ISS 12) and Spain (UB 38). Lack of funds postponed the formulation of recommendations to standardize the national systems for surveillance of canine leishmaniasis and the method of molecular characterization of *Leishmania*.

In M37-48, disease prevalence surveys were completed in Greece as well as by the designated partners (France, Spain, Italy, Hungary and Turkey), based on questionnaires to veterinarians' clinics, and focusing on follow-up mailings and telephone calls. However, surveys carried out in Hungary were not standardized, and this should be rectified in the last period of the project.

In 2008, work was to have focused on data processing and analysis. However, the long-term illness (cancer) of the PI of partner LSHTM 36 and the terminal illness and death of a son of the SP co-ordinator resulted in less progress with analysis than anticipated. It was only in September 2008 that approval to recruit a research assistant to help LSHTM 36 was granted and a suitable assistant identified.

A workshop was held in Granada, Spain (month 44), attended by all partners except UM1, from which it was clear that partners CNM 9, ISS 12 and UB 38 did not have adequate support from local geographers.

Description of work

In M48-60, the focus will be on data processing and analysis. This will be co-ordinated by NHM (with one PhD) and LSHTM (with one part-time post-doctoral geographer and one part-time research assistant). There are now plans to assist the teams without geographers (see 1.4.1.3. above; CNM 9 was assisted by a visit from LSHTM 36 and NHM 34 in December 2008). New vet questionnaire surveys will not be encouraged.
Deliverables Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project. D LEI10 – Descriptive analysis of spatial changes of prevalence rates in all 9 study regions in the past 20 years, after a new timetable has been agreed at EDEN AGM 2009 (M67). D LEI11 – To try to standardize the national systems for surveillance of canine leishmaniasis and the method of molecular characterization of Leishmania, after a new timetable has been agreed at the WorldLeish4 Congress in India (M67), which will be attended by key partners: UM1 39, ISS 12, NHM 34 and LeishRisk project. D LEI21 – Spatial analysis of prevalence rates in all main study regions in the past 20 years, after a new timetable has been agreed at EDEN AGM 2009 (M51).
Milestones and expected result M LEI17 - Preliminary spatial model outputs are available and allow for 1) first spatial analysis of disease / vector/ environment associations in at least one of 3 detailed study regions (M17), and 2) extrapolation to other regions (M54). M LEI29 - New timetables for completing deliverables to be agreed at EDEN AGM 2009 (M51). M LEI30 - Progress on completing the 3 deliverables will be reported by the SP co-ordinator to the Steering Committee meeting in May 2009 (M55). M LEI32 – Timetable of visits for data analysis and collaborative preparation of publications (M53): to be agreed after EDEN AGM 2009 (M51) and WorldLeish4 congress in India (M52).

WP LEI 5 – Data management and cross disciplinary modeling

Work package number	LEI WP5	Start date or starting event:				49
Participant id	CNM	ISS	IHMT	NHM	EUMS	LSH&TM	SZIE
Person-months / participant	1	2	0.5	1.5	2	1.5	1
Participant id	UB	UM1	UoC				Total
Person-months / participant	1	0.5	2				13

Objectives

The general objective is to generate current disease and vector risk maps on the basis of statistical models using pan-European remotely sensed and ground measured eco-climatic predictor data sets.

The specific objective is to collate all geo-referenced databases, and to analyse them to associate the distribution of leishmaniasis in the study regions with climate & environmental descriptors and the distributions of leishmaniasis and sandfly species. In months 25-42, the SP and HITS will collaborate more fully for some preliminary regional and Europe-wide modelling.

Work performed during previous reporting periods

In the first two years of EDEN (2004-6), we started multivariate analyses of the geo-referenced databases (WP1-4) and the modelling of the data from one of the three detailed study regions (in the French Pyrenées), to establish any significant associations (spatial and temporal) between leishmaniasis prevalence, sandfly vector densities and climate & environmental parameters. At the end of these preliminary studies, integrated activities with HITs started to be planned. Most teams attended workshops for the manipulation of datalayers with ArcView software.

In 2006-7, additional GIS training was provided for those teams seeking it (Barcelona, M30). New spatial analyses were restricted to 1) sandfly density or absence/presence in France in relation to climate (NHM 34 with LR Spatial Modelling HIT) and environment (NHM 34 with Environmental Change HIT), and 2) prevalence of canine leishmaniasis in relation to climate and environment (LSHTM 36). Canine leishmaniasis was not associated with vector density or absence/presence. Collaborative research was started with the Environmental Change and Modelling HITs, working with sandfly and environmental data from France, including analysis and planning sessions in Antalya (M27), Leuvan (M30) and London (M36). Progress was with other partners at AGM 2007 (M27), Barcelona workshop (M30), Piacenza workshop (M31) and AGM 2008 (M39).

In the period M36-48, the aim was to build on the collaborations started with the HITS to develop models of the spatial & temporal associations between leishmaniasis prevalence, its vectors, and changes in climate & environmental descriptors. Sandfly and canine leishmaniasis data for France (partner NHM 34) was given to Mathematical Modelling HIT in January 2008, and further sandfly data provided in September 2008. Preliminary results and conclusions have been distributed.

Description of work

In the next period (M49-60), the focus will be on collaborations with the HITS to develop models of the spatial & temporal associations between leishmaniasis prevalence, its vectors, and changes in climate & environmental descriptors. The models will be both statistical (in collaboration with LR Spatial Modelling and the Environmental Change HITs) and biological (in collaboration with the Mathematical Modelling HIT).

For the mathematical modelling of the French data started in 2008, a new timetable is required and a geographer identified to provide all the necessary datalayers.

Deliverables

Access will be restricted at this stage to members of the sub-project and associated HITs. It will be extended to the public before the end of the project.

D LEI12 – Models of the spatial & temporal associations between leishmaniasis prevalence, its vectors, and changes in climate & environmental descriptors, for three or more of the European regions studied in depth (e.g. southwest France, central Spain, northern Italy) (M60).

D LEI19 – Europe-wide model of the spatial association between leishmaniasis prevalence, its vectors, and climate & environmental descriptors (M60).

Milestones and expected result

M LEI29 - New timetables for completing deliverables to be agreed at EDEN AGM 2009 (M51).

M LEI30 - Progress on completing the 3 deliverables will be reported by the SP co-ordinator to the Steering Committee meeting in May 2009 (M55).

M LEI32 – Timetable of visits for data analysis and collaborative preparation of publications (M53): to be agreed after EDEN AGM 2009 (M51) and WorldLeish4 congress in India (M52).

Workpackages WNV1 to WNV5: West Nile virus

WNV personnel exchanges planned in the next reporting period include:

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Exchanges will be discussed at Annual Meeting. The objective will be to energize teams to get all their data into a common data format; for several teams this is now an urgent matter. An ideal strategy would probably be for each team to appoint a person responsible for the data in one or more work packages, and for those persons to meet to co-ordinate their efforts in the presence of a data manager and the sub-Project leader. Decisions will be made at the Annual Meeting.

WP WNV 1 – Landscapes, biotopes and habitats

Work package number	WNV1	Start date or starting event:			49			
Participant id	IZS	DDNI	NIRDMI	EBD	EID	IVB	CIRAD	Total
Person-months / participant	4	4	4	4	4	4	4	28

As explained above, it is important to repeat the same procedures, preferably by the same persons, over several seasons.

Transmission of West Nile virus is rarely evident in Europe. For this reason, it was always clear that evidence of transmission would be sparse at best, and it was unlikely that we would have any discernable outbreaks. As mentioned in the overview, there were only three confirmed human cases in the whole of Europe in 2007.

The concept of the project is to gain what little evidence we can of transmission across the geographic span of Europe (from SW Spain to the Black Sea) and look for evidence of environmental factors that might be associated with that transmission. For this reason, it is *not* impossible to project the same numbers of PMs per year. On the contrary, it is in nature of field work that several years data must be accumulated in the same manner; in the United States, I have heard of projects of this kind that spanned ten years.

Objectives

The fundamental approach of EDEN-WNV is gain insight into the variables favourable for epizootic/epidemic transmission of the virus through a systematic comparison of environmental parameters in all the study areas (the « comparative approach » presented in the original proposal).

Work performed during previous reporting periods

All participants have now gathered an impressive compendium of historical and environmental information pertinent to their study areas. Much of this is available in great detail and covers an extended time span (nearly 600 years for one site in Czech Republic!). The number of records of WNV in Romania over the past half century is particularly impressive, and, combined with serological data, detection of virus in birds and mosquitoes, and clinical records points to the Romanian study sites as having the highest level of epizootic transmission. It appears that this is only matched by enzootic/epizootic transmission in Spain, though human infections are less common. It is therefore highly desirable that NIRDNI and EBD make a special effort to co-ordinate their efforts in the context of WP1 and WP5. That is not to say that IZSAM/ISS and IVB data has less importance; on the contrary, their large databases, both in the field and the laboratory, will be a key component in the final, comparative analysis.

Ecological mapping surveys were pursued in the different countries and study sites, both for birds and mosquitoes. See section 2 'Work package progress of the period" in this report for more details.

Significant additions were made to the database. For the purposes of EDEN this should now be regarded as complete, although some teams may add further information

Description of work

The paramount task is to complete the consolidation of the data into the common database. Some teams have made good progress, others need to make an effort to catch up. This task will be emphasized at the Annual Meeting. The experience of CIRAD and DDNI should be shared with the other teams.

Deliverables

D WNV-23 – A complete database on biotopes and habitats collected during the project, plus a compendium on historical material where this is of interest.

D WNV-16 – Significant advance in ecological mapping and modelling (75% achieved). with good coordination between all partners (M67).

Milestones and expected result

M WNV18 – An array of six GIS models (Spain. France. Italy. Czech Republic. Romanian Plain and Danube Delta in an encouraging stage of completion (M60).

M WNV 21 A completed foundation for all milestones in WP 5, plus a publication on history of WNV in Romania (NIRDMIC). This will be the basis of a publication on the history of WNV worldwide.

WP WNV 2 – Vector bionomics and competence

Work package number	WNV2	Start date or starting event:		49			
Participant id	IZS	DDNI	UNISAP	NIRDMI	CNM	EID	IVB
Person-months / participant	4	4	-	4	4	4	4
Participant id	EBD	IPP	ISS				Total
Person-months / participant	4	4	4				36

Objectives

The principal objective of WP WNV2 is to identify potential WNV vectors (mosquitoes) in Europe, obtain maximal information on their ecology and seasonal activity, assess whether ectoparasites of resident and migrant birds may serve as reservoir of virus between mosquito seasons, and determine whether WNV is able to over-winter in Europe.

Field science requires much more time than laboratory science! Arbovirus transmission is seasonal and a useful data set can only be gained with several season's work. This is particularly true for EDEN because we seek associations between environmental change, such as climate; signatures of such associations can only be obtained by comparing incidence between years.

Work performed during previous reporting periods

Seasonal abundance was monitored by regular trapping of mosquitoes in Camargue (France), urban and peri-urban Bucharest and Danube delta in Romania, La Donna National Park (Spain), Czech Republic, Italy. Traditional entomological parameters have been recorded (longevity of females evaluated by ovarian dissection, identification of blood meals…). Some of theses samples were already tested for flavivirus/WNV by RT-nested-PCR but no isolation yet. Ectoparasites were collected on from birds trapped for ringing (WP WNV 3).

Participants had to tackle a plethora of preparatory tasks and problems: permission to work in conservation areas and to bleed wild birds, administrative requirements of parent establishments and national governments, formal agreements with non-EDEN groups working on WNV in the same regions, several others including new emerging diseases (avian influenza…).

Important additions were made to the data collections; teams have become more proficient with experience of previous years, and efforts made by EID and DDNI PhD students have been particularly fruitful.

Description of work

As in WP1: database and analysis are priorities, though some teams will continue collections. Communication between entomologists will be paramount.

Deliverables

D WNV-18 intensive collections of mosquitoes for at least 10 consecutive nights (two weeks) at three points during the mosquito season by CO2-baited and bird-baited traps (completed).

D WNV-19 Aspirator collections of mosquitoes attracted to horses during mosquito collection weeks (M41 – M47)

D WNV-20 Collections of ectoparasites associated with birds—resident and migratory—captured for serosamples (M40 – M48)

D WNV-21 Capture-mark-release of over-wintering *Culex pipiens* populations (M37-M40; M47-M51)

D WNV-22 Screen tests for virus in mosquito and ectoparasite collections by RAMP; confirmation of positives by virus isolation (M41 – M48).

D WNV-24 Article that includes seasonal fluctuations of all species captured (M67)

Milestones and expected result

As WP 1. Data collated into database. Data analysis for individual teams and for all teams involved. Individual publications and at least one publication that compares and contrasts the seasonal fluctuations at all sites for each year of collections. Publications describing findings of the project.

M WNV5 - Seasonal profile of adult mosquitoes. biting activity and host preference in study areas (M60)

M WNV6 - Abundance of adult mosquitoes in the context of climatic variables (M60). These may give clues to the likely impact of long term climate change on transmission.

M WNV7 - Evidence for/against role of ectoparasites in WNV transmission (M60).

M WNV8 - If there is evidence of current WNV transmission (seroconversion in birds. or horses. or clinical cases in horses or humans). pools of mosquitoes tested for virus (M60).

M WNV9 - Winter survival rates of mosquitoes and WN minimum infection rates (MIR) will be key parameters in models of epizootic transmission (M60).

WP WNV 3 – Public health and human activities

Work package number	WNV3	Start date or starting event:	49			
Participant id	NIRDMI	IVB				Total
Person-months / participant	-	-				0

Objectives

A serosurvey of the human population is highly desirable, but requires complex legal procedures and an immense amount of effort. Nevertheless, it is clear that seroprevalence is a critical parameter for assessment of the true public health significance of the virus.
Work performed during previous reporting periods The review of historical information on incidence/prevalence of WNV in humans and horses has been very instructive. Excellent data are now available for most of the teams. Seroprevalence in horses are continuing under WP WN 4. A serosurvey of the human population is much more difficult, however, because it requires complex legal procedures and an immense amount of effort. Useful data on seroprevalence in horses has been obtained for several countries, including the 2008 outbreak in Italy, but human serosurvey is abandoned. Exception would be if there is a sizeable outbreak. **Description of work** Serological tests on material collected by veterinarians should continue.
Deliverables This workpackage is terminated, except for further serological tests on horse blood collected by veterinarians. Exception would be in the event of a sizeable outbreak
Milestones and expected result

WP WNV 4 – Animal reservoirs

Work package number	WNV4	Start date or starting event:					49
Participant id	DDNI	IVB	CNM	IZS	CIRAD	UNISAP	EBD	NIRDMI
Person-months / participant	10	4	2	5	4	-	10	6
Participant id	IPP							Total
Person-months / participant								41

Objectives The general objective of this WP is to clarify the role of wild birds in enzootic/epizootic transmission of WNV in Europe by testing resident and migrant species, and domestic species. Also to determine seroconversion rates in horses. During the next 18 months, the objectives are to: • Continue to determine seroprevalence of WN virus in resident and migratory birds, and in horses. Species with particular interest will be at the discretion of local ornithologists after communication with ornithologists in other participating groups. • Continue to monitor infection rate by seroconversion in juvenile birds, recaptured birds, and repeat sampling from horses
Work performed during previous reporting periods (a) Seroprevalences in birds: Trapping of birds was conducted in France, Romania, Spain, and Czech Republic and Italy. A sampling strategy was developed to maximise the number of samples, optimize the range of habitats, and optimise comparative studies; a limited number of representative species was selected. Significant number of birds were captured and sampled for serology. Problems in France with diagnostics based on

ELISA screening summarized in Section 2.

Additional information: i) some teams had problems in getting the necessary permits for access and work in protected area and on protected species. ii) The outbreak of H5N1 influenza virus in many countries, activities were made more difficult and for example in Romania, bird sampling has been put on hold.

Field studies were conducted in Spain, France, Italy, Czech Republic and Romania. Results indicated a very limited WN transmission, except in Romania.

(b) Seroprevalence in horses

Seroprevalence surveys in Romania indicate a high transmission rate in the region adjacent to the Danube Delta. Significant transmission in the Bucharest area is also clear. In Italy, the results also indicate the circulation of the virus.

The same findings were made as for bird field studies, with high transmission rate in Romania, and low or no transmission elsewhere.

Probably the best year yet for serosurveillance of birds, and several articles already published. High seroprevalence at the extreme west (EVB) and east (DDNI and NIRDMIC) is circumstantial evidence of importance of migratory routes.

Description of work

Some teams will continue serosurveillance as part of projects funded from other sources.

Deliverables

As WP 1, but with emphasis on data processing and publications, both by individual teams and as joint publications between teams.

D WNV-11 Reports on seroprevalence of WN antibodies by species. sex. age. habitat and season. (M48)

D WNV-12 Reports on seroprevalence and sero-conversion rates in horses (M67).

D WNV-13 Completion of data and analysis of seasonal variations in composition and abundance of the avifauna in selected areas (M67).

D WNV-14 Further data and analysis of the role of birds in introduction and diffusion of the West Nile virus (M67).

D WNV-25 Publications describing findings of WP4 (M67)

Milestones and expected results

M WNV12 - Data over four seasons will complete the study of host species (M47).

M WNV13 - Comparison of species data with mosquito capture data within and between study regions will complete the study of bird/mosquito species involved in transmission. the habitats/mosquitoes associated with this transmission. and regional differences in transmission dynamics (M47).

M WNV14 - Seroprevalence and seroconversion rates. combined with seasonal information from mosquito captures. will further contribute to understanding of seasonality of transmission. which in turn may help interpret impact of variations between seasons. and indicate likely impacts of future climate change (M47).

M WNV16 - Seroprevalence in migratory birds will further contribute to identification of species likely to be involved in intercontinental transport of virus. and may provide clues as to contact points between migratory and resident species (M30).

M WNV20 - Data collected during the first seasons will contribute to epidemiological modelling and analysis (M47).

WP WNV 5 – Data management and cross disciplinary modeling

Work package number	WNV5	Start date or starting event:			49			
Participant id	IPP	IVB	EID	CNM	IZS	CIRAD	NIRDMI	DDNI
Person-months / participant	2	2	2	1	2	2	2	2
Participant id								Total
Person-months / participant								15

Objectives

The general objective of this WP is to integrate information gathered through historical time series analysis and high resolution epidemiological studies in WP1-2-3-4 in order to construct area-wide statistical models for the occurrence of WNV in Europe. Now that a fair amount of data is available, more attention will be paid to this WP. During the second 18 months the specific objective will remain to be to integrate all collated data collected during the first two seasons. Preliminary epidemiological analysis outputs will allow the proposal of pathways for future modelling exercises and the improvement of field sapling protocols.

Work performed during previous reporting periods

Nearly all the teams have produced voluminous quantities of data, nearly all of which is in a form that must be processed before meaningful epidemiological analysis. As soon as Elisa- results are available, preliminary epidemiological analysis outputs will allow the proposal of pathways for future modelling exercises and the improvement of field sampling protocols. At this stage, maps of ecological units of relevance for mosquitoes and birds communities have been drawn up, and in coherence with the ability to discriminate these units from satellite images, and retrospectives populations of mosquitoes and birds have been evaluated. The diversity of the associations between reservoir and vector in space and time are explored. The approach his now well designed and has been developed in France, Italy and Romania.

Substantial work has been accomplished to model vector abundance in Camargue, in collaboration with MAL and HIT HRRS. Several papers were published in good journals.

A mathematical model of WN transmission was developed in collaboration with Utrecht University and IVB. The works was published in a good journal.

Collaboration between CIRAD, DDNI and IZS has been fruitful.

Description of work

WP 1 and WP5 must be the principal activities of the sub-project. A viable game plan must be worked out at the Annual Meeting. In addition to analysis achieved within the timeframe, partners will be encouraged to seek funding for futher studies based on the foundations created by the EDEN project. In addition to professional publications, teams are encouraged to seek ways to inform the public in their respective countries on the results of their studies.

Deliverables

A comprehensive database of all material collected during the project. Data analysis in collaboration with the HIT personnel. Foundations for futher studies, post-EDEN.

D WNV-15 Preliminary epidemiological analysis of the first two complete transmission seasons (2006 and 2007) (M60).

D WNV-26 Publications describing findings of WP5 (M67)

Milestones and expected result

M WNV17 - Data collected during in the first 36 months will contribute to epidemiological modelling and

analysis. This will be critical for planning of the next 18 month period (M42).

Workpackages MAL1 to MAL5: Malaria

WP MAL 1 – Landscape, biotopes and habitats

MAL personnel exchanges planned in the next reporting period include:

NO exchanges are planned. However most of the partners will meet at the 5th international Sove congress "VECTORS WITHOUT BORDERS" 11th-15th October 2009, Belek-Antalya-Turkey. We plan to have an special SP Mal Eden meeting at that time.
Scientists from Morocco, Algeria, France and Spain Malaria SP will participate to a workshop organised by HIT LRRS (Annelise Tran, Cirad) in Montpellier in July 2009 entitled “Associations between entomological data and environmental data”

Work package number	MAL1	Start date or starting event:			49		
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	2	1	2	2	2	2	3
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	0	4	3				21

Objectives

The general objective of this WP is to characterise at a high resolution (30m), dominant ecosystems and environmental parameters linked to Anopheles vectors. During the next 18 months, the scientific objective include further improvements in the landscape description of the model regions, in secondary model areas and in other relevant Mediterranean ecosystems

Work performed during previous reporting periods

Model regions have been selected and d in every country: Algeria (Ouargla), France (Camargue), Italy (Maremma), Morocco (Province of Chefchaouen), Portugal (Troia Peninsula), Romania (south of Bucharest), Spain (Ebro Delta), Turkey (Sanlıurfa province). Additional areas have been selected in several countries: Algeria (El Tarf), France (Corsica), Italy (Sardinia, Po Delta and Sicilia), Morocco (Larache), Romania (Danube Delta), and Spain (Albufera and Murcia regions). Vector and Human host habitats have been described in the studied areas and climatic data have been extracted at a regional basis. The first GIS version are available for most of the countries and some analyses between climate data and mosquito densities (based on historical data set) have been proceeded. First generation of models has been finished notably in France, Portugal, Algeria and Turkey.

Year 4: Draft of environmental map of model areas related to anopheles breeding sites in model areas have been under construction in first model area in France, Algeria, Portugal, on going in Romania, Spain, Turkey; Italy and Morocco

Reports on environmental parameters linked to anopheles vectors and malaria parasites in the second model areas have been initiated in Corsica (France), El Tarf (Algeria), Danube Delta (Romania) and in Larache (Morocco). See report WP MAL 37-48.

Description of work

Algeria: During the period (1/11/07 to 31/10/08), the final improvements consisted to the description of the landscape, biotope and habitat of the two model regions (El Tarf in north and Ouargla in south of country) were done. In addition an extended area, Tinzaouatine, a small village close the Algerian-Malian border (19°.95’ N; 02°.95’), in where an outbreak of malaria appeared in 2007, was described. For more details, a satellite image was provided by the Algerian Space Agency (Fig1).

The effects of eutrophication growth on malaria vector abundance were assessed in the National Park of

El Kala between April to November 2007, and in the summer 2008. Despite the eutrophication of the Lake, Anopheles labranchiae is still present in this area producing in the end of summer an important anophelism. The vectorial capacity exhibited a seasonality variation. However, the intensive eutrophication may have a negative impact on the population dynamics of An labranchiae in the future. For the last 12 month two papers on environmental changes and malaria resurgence, the first one related to Ouargla and the second for El Tarf areas will be submitted. Spain: Integrate the entomological data (WP2) and human characterization analysis (WP3) from the Ebro Delta in the GIS in order to obtain the risk model. To compare the climodiagrams from the three analyzed regions and obtain the Index of Potential Transmission of Malaria for Albufera and Murcia region in order to compare with the index of the Ebro Delta. France: All data obtained from Corsica will be analysed, an article must be write.
Deliverables D MAL01 – Report on environmental parameters linked to anopheles vectors and malaria parasites, particularly in the second model areas (M60). D MAL02 – Spatial database on environment in every selected area (M37 – M42 – M60). D MAL03 – Standardised description of environment and climate into the model areas (M37 – M42 – M60). D MAL04 – Draft of environmental map of model areas related to anopheles breeding sites in model areas (M37 – M42 – M60).
Milestones and expected result M MAL04 – Collected raw data from first model areas is available and enable to create spatial data layers to be included in the GIS (M 37). M MAL05 – Preliminary GIS model enable first spatial analysis of vector / Malaria/environment associations only in some partners, other ongoing (M37 to M48). M MAL17 – Collected raw data related to second model area are available (M48). M MAL18 – Detailed environmental information about second model areas is available (M48). M MAL 28 – articles, or at least reports on mosquito environnement, available (M60)

WP MAL 2 – Vector bionomics and competence

Work package number	MAL2	Start date or starting event:				49	
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	6	2	5	5	5	3	10
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	1	15	6				58

Objectives The general objective of this WP is to study the population ecology of anopheline larvae and adult populations in Europe in order to estimate the mosquitoes' vectorial capacity and competence. During the second 18 month period of the project the specific objectives are: • To complete the recording of historical data on the distribution and bionomics of anopheline species; • To conduct population dynamics studies; • To continue the vector competence studies on potential vector; • To characterise species and populations in the Anopheles maculipennis, An. claviger, An. sergentii

and An. hyrcanus complexes and their distribution.

Work performed during previous reporting periods

A review of the literature and historical reports from Ministries of Health, research institutes and mosquito-nuisance control agencies was conducted to obtain historical data on the distribution and bionomics of anopheline species, in each partner country. Common protocols for (i) sampling mosquitoes, (ii) testing *Plasmodium* mosquito infection and identifying blood meal and (iii) morphological and genetical identification of species/species complexes in the model areas have been provided. Mosquitoes have been collected by various methods depending on location, mosquito biology, and feasibility; A huge amount of data on all these population biology parameters is now available. Multiplex PCR for identifying species from Maculipennis complex has been improved and evaluated in different countries. Several rDNA sequences from specimens from *Maculipennis* complex from different countries have been obtained. Morphometric analysis of *An. Hyrcanus* has been conduced, a paper has been in preparation. First result of vector competence of anopheline populations from Portugal, Turkey, France, Algeria, Italy, Morocco has been assessed with Nijmegen partner, this study continue with Pasteur Institut in Paris. Only *An. hyrcanus* from France was unsessfully infected. Several articles have been published. A review of the literature and historical reports from Ministries of Health, research institutes and mosquito-nuisance control agencies was conducted to obtain historical data on the distribution and bionomics of Anopheline species, in each partner country. Mosquitoes have been collected by various methods depending on location, mosquito biology, and feasibility; a huge amount of data on all these parameters is available.

Year 4: The transmission of the African NF54 Plasmodium falciparum strain has been experimentally initiated with a new partner: Pasteur Institut. Two colonies were tested: An labranchiae from Corsica (France) and from Italy. This collaboration will continue next year with other EDEN partners.

Specimen of anopheles Labranchiae from Italy, Maroc, Algeria and France was processed using microsatellites marker, a paper is in preparation. A study on genetic structure of An. atroparvus population is on going. Morphometric analysis of An. Hyrcanus has been almost finished. See report WP MAL 37-48.

Description of work

Algeria: For the study of the susceptibility of the An labranchiae to the NF54 p falciparum twelve anopheles blood-fed were dissected, three (35%) have developed oocysts. The experimental infection of An multicolor will be attempted in the next year at the Institut Pasteur of Paris

Morocco: To analyze field collected data (molecular identification of complex species by PCR)

Spain: To compare the results of the studies developed in the Ebro Delta (2005-2006 and 2008) and try to determine the possible correlation with climate conditions.

Collaboration with Hygiene and Tropical Medicine Institute from Lisbon (Portugal) in order to obtain an experimental colony of An. atroparvus from Ebro Delta; to determine the presence or absence of pre-gravid females (fact found in atroparvus females from Portugal); to determine the rate of insecticide resistance to the authorized insecticides; and to obtain a resistant phenotype of An. atroparvus.

Study the An. atroparvus resistant phenotype by RNA extraction and sequencing of the KDR gene in order to determine the possible mutations in this gene.

Contact with the Pasteur Institute to test the degree of susceptibility of An. atroparvus from the Ebro Delta to the different Plasmodium species.

France: Analysis of all anopheles specimens will be processed. Results of the study of genetic structure of An. labranchiae will be published.

Deliverables

D MAL05 – Update of past and current anopheline species in selected model areas and in every partner country (M37 and M42).

D MAL06 – Update of the database on anopheline collections in model areas (M37 – M42 – M54).

D MAL07 – Report and scientific articles in international journals on biology and vectorial role of

anopheline populations from model areas, including An. plumbeus (M37 – M42 – M48). D MAL08 – Report on anopheline systematics (M30) and population genetics (M67). D MAL09 – Report on vector competence of mosquito populations and species not yet tested (M37 and M67).
Milestones and expected result M MAL08 – Collected raw data (including archived data and fieldwork in spring-summer 2005 to 2007) to create Anopheline data layers (for partners who have not finalized yet) (M42). M MAL09 – Spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the model areas (M48). Completed in first model area in France, Algeria, Portugal; almost finished in first model area in Turkey; Morocco, Italy, on going in Romania, Spain; Ongoing in second model area in all country where such an area is selected. M MAL19 – Data on the biology and the distribution of *An. plumbeus* (M48). Only France has collected data. Very few specimens (larvae) have been collected. No more investigation is planned on this time consuming subject. M MAL20 – Infection rate of natural anopheline population in Model areas in Algeria, Morocco and Turkey (M48). Done in Algeria and Morocco (all mosquitoes negative). Planned in Turkey M MAL21 – Vector competence for populations of Anophelines in countries not tested yet (M 48). Spain, Algeria and Romania, and more populations from Corsica, M MAL22 – First results on population genetics of *An. maculipennis s.l.* (M42). Mosquito already processed. Articles need to be written M MAL23 – Update of distribution of Anopheline species in partner countries (M48). M MAL 26 - First scientific publications on mosquito biology in international journal (M48) : done

WP MAL 3 – Public health and human activities

Work package number	MAL3	Start date or starting event:			49	
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	3	1	3	2	2	1	3
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	0	3	3				21

Objectives The general objective of this WP is to understand area-wide historical malaria incidence and risk patterns in relationship with recorded public health activities and surveillance, warning and control campaigns and changing human movements patterns and activities. specific objectives of the next 18 months period are: To evaluate the impact of public health activities, human movements, and human activities, on malaria incidence and risk. To record and analyse past and present data on malaria incidence and the impacts of surveillance, warning and control campaigns.
Work performed during previous reporting periods A review of literature and historical reports from Ministries of Health, research institutes and malaria control agencies has been realised in every country to obtain historical data on malaria in Maghreb and Europe. Collection of recent data on malaria incidence (autochthonous and imported) in Maghreb and Europe has been already obtained or initiated in every country. These data suggest that imported malaria might be underestimated in several countries. Public health activities related to malaria have been listed in every country, and their impact on malaria

incidence will be evaluated in model areas where malaria used to occur. Studies on the impact of past and current human activities (modifications of agricultural and/or sociological practices, land use change; irrigation; deforestation; dams; urbanisation; cattle breeding; etc) has been conducted in almost all partner countries. Year 4: Collection of recent data on malaria incidence (autochthonous and imported) in Maghreb and Europe continue. The data obtained by questionnaires and mapping in 2007 must be submitted for publications. See report WP MAL 37-48. **Description of work** **Algeria**: Two papers will be submitted in 2009: Malaria in the Algerian Sahara submitted to the bulletin de la Société de Pathologie exotique, and the second paper "Malaria outbreak and first report of Anopheles gambiae s.l. in South Algeria is in preparation and will be submitted to the revue emerging of infectious disease . **Spain**: Determine the representative characteristics of the residents and tourist of the Ebro Delta. Define the different behaviour patterns of the people that live or visit the Ebro Delta (For example: activities, use of protection or job) and according to the entomological results of WP2 determine what of these patterns would be the most susceptible. **France:** The data obtained by questionnaires and mapping in 2007 must be submitted for publications.
Deliverables D MAL11 – Report on human movements in relation to malaria importation/exportation in every 8 "field" country, and particularly in model areas (M18 – M42 – M67) D MAL12 – Report on the relationship between modifications of environment/human activities and malaria/potential vectors in model areas (M67)
Milestones and expected result M MAL11 – Complete data on autochthonous and imported malaria incidence in the 8 "field" countries, including data from the model areas (M30-M42) M MAL12 – Complete collected raw data (including archived data and fieldwork in spring-summer 2005 and 2006) to create public health – human activities layers –particularly in malaria endemic countries) (M30-M42) M MAL25 – Data on human behaviour related to mosquito and malaria (M42) M MAL 30 – articles, or at least reports, on KAP surveys, as well as malaria epidemiology in Algeria and Turkey (M60)

WP MAL 4 – Animal reservoirs

Not relevant

WP MAL 5 – Data management and cross disciplinary modeling

Work package number	MAL5	Start date or starting event:				49
Participant id	IRD	EID	HUESRL	ISS	UVEG	IHMT	INH
Person-months / participant	3	1	3	3	2	2	3
Participant id	NHM	NIRDMI	IPA				Total
Person-months / participant	0	3	2				22

Objectives
The general objective of this WP is to model the risk of malaria transmission and spread in Europe and

the likely impact of environment, human behaviour and climate changes using data generated by WP1-2-3. specific objectives of the next 18 months are:

- To collate climate, hosts' behaviour & environmental descriptors in relation with the distribution of malaria vectors and cases.
- To model the distribution of vectors, the risk of transmission and spread

Work performed during previous reporting periods

During year 1 data has been collected, collaborators identified and trained. Workshops on modelling and data management have been organised. During year 2 and 3 data collected in WP 1, 2 and 3 have been introduced into geo-referenced databases for further analysis. Data collection including Remote sensing data and derived parameters will continue Y4.

Data collected in WP 1, 2 and 3 have been introduced into geo-referenced databases for further analysis.

Year 4: Data collection including Remote sensing data and derived parameters will continue Y4. Collaboration has been established between HR HIT team and Morocco, and between modeling team and France and Turkey. First entomological risk models have been established by some partners (France (published) and Turkey). See report WP MAL 37-48.

Description of work

Portugal: Next month's we will accomplish the vector capacity, susceptibility and transmission risk maps. Then we should be able to proceed with cellular automata and multi agent simulation of malaria spreading scenarios.

Spain: Collaboration with the horizontal teams to develop the model risk of Malaria transmission in the Ebro Delta

France: A R0 mathematical model will be developed in collaboration with HIT modeling team (Heesterbeek group)

Deliverables

D MAL13 – Complete (or develop when not done) the first draft of geo-referenced database on environment, vectors, malaria, and public health (M30 – M48 – M60)

D MAL14 – Complete (or develop when not done) first draft of maps and risk models in selected areas (M18 – M48 – M67)

D MAL15 – Finalized bibliographic database on malaria and vectors in Europe (M60)

Milestones and expected result

M MAL13 – Spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the 8 model areas (M30 to M60).

M MAL14 – Preliminary spatial model allowing first spatial analysis of vector/ malaria/ environment associations in the secondary model areas (M30-M42)

M MAL15 – Development of a common database to all malaria SP partners (M48).

M MAL27 – First scientific publications on modeling in international journal (M42).

M MAL 31 – articles available, or at least reports, on model and distribution maps, (M60)

Workpackages AFR1 to AFR5: Africa platform

WP AFR 1 – Landscapes, biotopes and habitats

AFR personnel exchanges planned in the next reporting period include:
None.

Work package number	AFR1		Start date or starting event:			49	
Participant id		ISRA	IPD	CIRAD	IAV			Total
Person-months / participant		4	0	4	1			9

Objectives

The main objective of this WP to identify environmental parameters and events associated with documented RVF outbreaks in livestock (ISRA, CIRAD) and humans (IP) and WNV incidence in endemic areas, and select indicators for the monitoring of epidemiological processes (CIRAD) and emergence risk.

For the next 18 months, the specific objectives of this WP are:

- To finalize the review of RVF and WNF history in the endemic area;
- To integrate the regional database including RVF events and available environmental data;
- To describe the diseases patterns in the select study sites.
- To determine the factors (environment, serological, virological and animal mobility) that determine the conditions of emergence and spread of RVF in West Africa

Work performed during previous reporting periods

The main outbreaks of RVF in livestock from 1988 to now have been documented and imported in a database. The survey of cattle sentinel herds is on going and no RVF virus circulation is detected in the Senegal river basin during the present rainy season (from June to November) in Senegal. Serological results are pending. The prevalence of IgG WN specific antibodies in sampled horses from villages and markets of the Delta of Senegal River was high. Results from sentinel chicken reveal a low circulation of WNV around the national Park of Djoudj. In Morocco, Larache area, the seroprevalence reaches 57% in Equidae.

High and very high-resolution remote sensing images were used to provide ecological maps of the study sites in Senegal, the Senegal river valley (Landsat imagery) and the Ferlo region (Quickbird). Studies on the relationships between serological, entomological, wild bird data and land-cover are planned, as soon as the data are integrated in the EDEN-AFR database.

Year 3: ISRA team visited 8 sentinel herds during the first mission in early rainy season. Two hundred and thirty sera were sampled and tested for Rift Valley fever antibodies (IgG). All test results were negative. After the rainy season, 4 sentinel herds were sampled again (240 sera) and no positive result was observed. In total, 470 sera samples were thus tested and all were found negative to RVF. Therefore, for the current reporting period, no circulation of RVF virus was observed in the survey area. In addition, IPD has prepared a document summarizing previous RVF outbreaks in Senegal and neighbouring countries, as draft of a paper to be submitted for publication.

In Morocco, 240 sheep sera were sampled from 5 different regions. None tested positive for RVFV.

Sera samples were collected by IRD in migratory and local wild birds, and transmitted to IPD for the detection of WNV antibodies. The analysis process is pending. Preliminary results indicated a WNV seroprevalence of 1.4% in Barkedji, and 1.2% in the Djoudj park.

In order to set up sentinel horses to monitor West Nile virus circulation, 570 horses have been screened to identify negative individuals to be included in a follow-up survey. It turned out that most horse sera had WNV antibodies: only 38 negative individuals were identified and included in the longitudinal survey to monitor WNV circulation. This work was jointly carried out CIRAD and IPD.

IPD has conducted a phylogenetic study of the WNV strains isolated in Senegal and neighbouring countries. This study has confirmed that both WNV lineages I and II circulate in Senegal, and that lineage I shows an important genetic variability. A close relationship was found between Senegalese, Mediterranean and European isolates, thus providing further evidence that bird migrations may disseminate the WNV. Furthermore, a new lineage of WNV has been identified and its full length genome has been sequenced.

No further study was implemented to characterize landscapes and their changes (see report of the previous period).

No further study was implemented to characterize landscapes and their changes. Field studies went on to assess the seasons with a highest risk of WNV and RVF transmission.

Description of work

The existing databases on environment are being used by CIRAD to find the most favourable conditions for the transmission of WNV and RVF. This will be updated as data follow.

Deliverables

D AFR 02 - Description of WNF distribution in horses in the Senegal River delta and valleys and Morocco (M54) In progress

D AFR 04 - Spatial database of environmental information available from each study area (M48) Done

D AFR 15 - Extension of census of RVF outbreaks since the 80's in the region (Mali, Mauritania) (M48) Done

D AFR 16 - Landscape typology (M48) Done

D AFR 17 - Description of major breeding systems associated to landscapes (M48) Done

Milestones and expected result

M AFR03 - The serological analysis have been conducted and data are available for analysis of patterns (M48).

Serological analyses were done and results are available. For EDEN wild-bird data, serological analyses were done but results are still being analysed.

The assessment of the endemic stability of WNF in Senegal river valley will be studied in collaboration with the transversal team of disease transmission modelling. It is plan to model age-dependent force of infection from horse prevalence data harvested in 2005, 2007 and 2008. To test the endemic stability. Indeed, clinical disease seems to be scarce despite high level of infection what the sign of endemic stability is. These analyses would lead to publication in the end of 2009.

Combining use of satellite ETM+ images from the dry and wet seasons and specific statistical analyses using generalized linear mixed model and a component analysis strategy, horse data from 2005 provide interesting results which will be presented in the next SVEPM meeting (2009). These results showed that WN virus is endemic in this region; the transmission differed with landscape despite a global high transmission level. This first landscape approach in an endemic area may provide a methodology to identify risk areas in non-endemic areas and target the surveillance.

M AFR04 - Regional database on RVF events in domestic ruminants in Mali, Mauritania and Senegal is on the EDEN Web-site (M48).

A database is available but it needs cleaning.

WP AFR 2a – RVF vector bionomics and competence

Work package number	AFR2	Start date or starting event:			49		
Participant id	IPD	ISRA	IRD	IAV			Total
Person-months / participant	3	4	0	1			5

Objectives

The global objective of this WP is to document the role of local vectors that are involved in RVF transmission in epidemic areas (Senegal river basin) and the competence of widespread candidate vectors in presently disease free areas. This will enable to estimate risk of spread in case of introduction of the virus. For the next 18 months, the specifics objectives of this WP are:

- To finalize the description of arthropods populations (mosquitoes, ectoparasites) feeding on RVF sensitive vertebrate in and out epidemic area;
- To obtain other mosquitoes species bred in controlled conditions;
- To assess infective challenge and evaluate vector competence.

Work performed during previous reporting periods

In epidemic foci of RVF, the census of mosquitoes and ticks with potential role in diseases transmission is going on in different sites from contrasted ecozones in Senegal. Three sites are studied: Djoudj national park, Ross Bethio (ISRA) and Barkedji. Similar studies are implemented in Morocco. Collected mosquitoes are tested for virus isolation, and until now, no virus has been detected.

Aedes vexans, one of the RVF candidate vector has been successfully adapted to breeding conditions. Three strains of RVF Virus isolated from human, bovine and mosquitoes were selected for vector capacity trials.

Year 3: mosquito trapping has been continued in the 3 sites of the Africa platform (Barkedji, Ross Bethio and the Djoudj Park), with CDC light traps baited with CO2, goat or sheep-baited traps, and pigeon-baited traps. Different environmental units were sampled according to a study design defined together with the high-resolution remote-sensing horizontal team.

Collected mosquitoes have been identified and monospecific samples have been prepared for virological analyses. This work has been performed at ISRA, IRD, IPD, and IAV (Morocco). In addition, IPD has used active sampling methods (aspiration of engorged females) for blood meal studies.

In total, 5,056 mosquitoes belonging to 5 genera (Aedes, Mansonia, Culex, Aedomya, and Anopheles) and 14 species were sampled and pooled into 282 monospecific samples at ISRA. Five species (Culex tritaeniorhyncus, Cx poicilipes, Cx naevi and Mansonia uniformis) represented 95% of the mosquitoes.

At IPD, the figures were, with CDC traps, 14,630 mosquitoes in 7 genera and 38 species. During year three, Aedes (Ae.) vexans was the most abundant species (45.4%) compared to Culex (Cx) poicilipes which dominated in the year two. It must be outlined that these quite different species pattern between ISRA and IPD studies are related to the contrasted environments in which the two studies were carried out: Senegal River Delta for ISRA, and Ferlo Valley for IPD (Barkedji).

From the 34 pigeon-baited traps, a total of 920 mosquitoes were collected, 98.2% of which belonged to the Culex genus. Pigeon was the most attractive species compared to chicken, Cx naevi was the most abundant mosquito species at the tree canopy, while Cx poicilipes was more abundant at the ground level. Monospecific mosquitoes were also pooled for further analyses, as well as engorged females for blood meal analysis.

Candidate mosquito vectors have been successfully reared. However, biosafety conditions were not sufficient to start experimental vector capacity and competence studies. This situation will improve during the next reporting period.

At IRD, the sampling was performed in the ornithological park of Djoudj, using CDC light traps baited with CO2. In total, 573 monospecific samples were prepared for further virological analysis.

In Kenitra and Larache region (Morocco), mosquito sampling was performed with CDC traps in rice fields, permanent water pools, hen houses and stables. Preliminary results have shown the importance of 3 species: Anopheles labranchiae (33.36%), Coquillettidia richiardii (19.18%) and Culex theileri (43.62%). Monospecific samples were also made for virological research.

ISRA at the site of Ross Bethio has collected a tremendous amount of information by using different trapping methods. These indicate that associating a general trapping method such as CDC light trap with more specific host-baited trap give more precise information on the attractiveness versus feeding. Several species in some five genus have been routinely trapped but two majors species *Culex trinaeniorynchus* and *Culex naevei* have emerged in attractiveness as well as in feeding within an interesting period of the year thus giving majors trends in the host vector and period of risk scheme

The work reported this year in Morocco was done to investigate the role of migratory birds in the transmission of west nile virus. This work comes to complete previous works on horse (Iraqui 2006) and on mosquitoes (Ladib 2007). The method used is the RT PCR to investigate the presence of virus in the samples collected. Sixteen bird families have been tested and all were negative except for two: Phalacrocoracidae, represented by cormorans.

Description of work

Lots of information is yet to be included in the general round up to get more precise idea on how, once all these results have been put together, more light could be shed on the risk analysis. It is why lots of efforts will be put in sharing data for a more general use within the platform and outside but still within EDEN project.

Deliverables

D AFR 05 - Analysis of mosquito and ectoparasites census data in relation to their potential role in RFV epidemics (M67). This will be achieved as field data continue to follow.

D AFR 06 - Comparative study on the blood-feeding entomo-fauna in disease foci and in the northern potential sites (M67). A significant number of field data have been collected this will be achieved as they will regularly be sent to data management.

D AFR 07 - Rearing of first candidate mosquitoes (M37). Achieved. This has been solved by IPD.

D AFR-08 - Report on the vector competence of local and exotic species (M42). Not achievable for biosafety reasons.

D AFR-09 - Description of the impact of some abiotic factors on the vector competence (M42). Not achievable for biosafety reasons.

Milestones and expected result

M AFR05 - The list of candidate arthropods for RVF transmission has been up-dated (M42).

Achieved.

WP AFR 2b – WNV vector bionomics and competence

INCLUDE person-month inputs according to work planned

Work package number	AFR3	Start date or starting event:			49	
Participant id	IPD	ISRA	IRD	IAV			Total
Person-months / participant	0	4		1			5

Objectives

The global objective of this WP is to document the roles of local vectors, reservoirs and hosts and their capacity to transport WNV to Europe, directly or through relay areas. For the next 18 months, the specific objectives of this WP are:

- To finalize the census and to test wild birds in WNV endemic areas of Senegal and in Morocco ;
- To complete the collection of arthropods expected to be involved as vector and/or reservoir for WNV;
- To isolate WNV strains from vectors.

Work performed during previous reporting periods

Wild birds from selected areas (Parc National des Oiseaux du Djoudj and Ferlo Valley in Senegal, Marais de Loukos in Morocco) have been censed and sampled for WN serology. The database also includes data from other sources, notably from the Natural History Museum of Paris, where are recorded more than 1,140 migratory birds from Senegal or Europe since 1950. Blood samples from wild birds will be analyzed in the coming weeks. In addition, ectoparasites have been collected on these birds and prepared for viral analysis. Candidate mosquitoes for WNV transmission have been sampled with CDC light trap or pigeon baited trap (8309 mosquitoes, 37 species). Collections of ticks are undertaken monthly (from horse and bird nests).

Year 3: on the entomology side, the same work performed in the WP AFR 2a is still valid for this WP AFR 2b; differences will take place in the analyses made.

The work done by ISRA in the previous workpackage (2a) is valid for this section

Description of work

Comments in the previous section remain valid.

Deliverables

D AFR 10 - Report (list and census) on the migrant and resident wild bird species in selected areas (M48). Not achievable: see IRD comment on next page. However, the census and list of the migrant and resident wild bird species in selected areas is available and continuously updated following catches of new species.

Milestones and expected result

M AFR08 - Updated bird census is available (M48). Not achievable: see IRD comment on next page. However, the census and list of the migrant and resident wild bird species in selected areas is available and continuously updated following catches of new species.

M AFR10 - Serological analyses have been conducted and prevalence data in resident and migratory wild birds are available (M67). The work has already been done by IPD. Data are being processed and organised as publication, then discussed with IRD.

M AFR15 - WN virus strains have been isolated (M42) No virus isolated yet. Achieved. A new strain has been discovered as previously reported.

Explanations provided by IRD (partner n°13) for missing data to complete deliverables D-AFR-10, 21, and 24, and milestones M-AFR-08, and 18.

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A Monsieur le Dr Amadou DIAITE
Responsable du Plateau Technique EDEN_Afrique

Dakar, le 25 Juin 2009
Objet : Réponses pour le « Rapport Tuomo ».

Monsieur Amadou Diaïté

Depuis le début de l'année 2009, nous avons eu au moins trois réunions de concertation (deux a l'ISRA et une a l'IPD) pour résoudre les problèmes concernant les engagements qu'avait l'UR 178 vis-à-vis du Plateau Technique EDEN-Afrique. Les activités de l'UR 178 ont été suspendues après mon départ imprévu de Dakar en août 2007, cette UR à laquelle j'appartenais (avec G. Chauvancy avec moi à Dakar) est arrivée en fin de vie en décembre 2007 et rien n'a plus fonctionné du côté de l'IRD-Dakar en 2008 pour le programme EDEN-IRD au Sénégal.

Ces réunions ont eu lieu, car nous sommes tous conscients à Dakar (ISRA, EDEN, IRD, IPD), qu'il faut terminer avant la fin de l'année 2009 l'exploitation du matériel qui est resté cryopréservé à l'IRD. Depuis que je suis revenu à Dakar début 2009 (dans une nouvelle UMR), nous avons résolu ensemble (ISRA et IRD) le problème des prélèvements IRD-EDEN stockés depuis 2 ans dans un congélateur a moins 80°, c'est à dire :

- les sérologies d'oiseaux (volet IRD-EDEN), tous les prélèvements ont été transférés dans le Laboratoire d'Arbovirologie du Professeur Amadou Sall à l'IPD de Dakar,
- les moustiques capturés dans le Djoudj (volet IRD-EDEN), reste un problème d'identification concernant certains *Aedes*, c'est est en voie d'être réglé avec Laboratoire d'Entomologie du Professeur Mawlouth Diallo à l'IPD de Dakar,

Il faut maintenant analyser le « volet ornithologie » (volet IRD-EDEN) pour 2008, sur les études des voies de migrations d'oiseaux entre l'Europe et l'Afrique de l'ouest et concernant de nouvelles couches de migration à ajouter à la carte déjà en préparation dans les rapports EDEN (notamment celui de 2008). Ces données qui devaient être couplées avec les sérologies réalisées à l'IPD sur les espèces migratrices. Je ne possède pas d'informations sur ce volet ornithologie, car avant mon départ en août 2007 presque tout avait été délégué vers la France avec l'ornithologiste IRD Francois Baillon.

Cet ornithologiste qui était à l'IRD-Dakar pour le Plateau Technique EDEN_Afrique est parti en France fin 2006 (IRD-Orléans). Par la suite, concernant EDEN, à partir d'Orléans, F. Baillon avait des contacts et des réunions en 2007 (et 2008 ?) au CIRAD de Montpellier. Du fait de la disparition de l'UR 178 fin décembre 2007, je ne sais pas si F. Baillon, ornithologiste IRD (IRD-Orléans) a vraiment continué de travailler pour EDEN en 2008 et après.

Voici donc seules informations que je possède concernant ce « volet ornithologie » IRD-EDEN :

- 1// l'ornithologiste IRD F. Baillon avait en sa possession la base de données du Muséum MNHN sur le baguage des oiseaux en Europe et recapturés en Afrique de l'ouest. En 2006, à l'IRD-Dakar, G. Chauvancy avait commencé d'établir des cartes de migration d'oiseaux à partir de cette base de données. Fin 2006, F. Baillon a refait des cartes avec E. Etter (ISRA CIRAD-Dakar) et G. Chauvancy a donc arrêté ses cartes. E. Etter et F. Baillon étaient en relation (je crois) avec un ornithologiste du CIRAD. Ensuite, fin 2006, F. Baillon est parti de l'IRD-Dakar pour Orléans et E. Etter est parti de l'ISRA-Dakar mi 2008 pour aller à Montpellier (CIRAD),
- 2// G. Chauvancy avait fait par ailleurs (autrefois pour l'UR 178) des cartes avec les voies de migration intercontinentales des oiseaux, à Dakar, ces cartes ont été transmises à V. Chevalier vétérinaire CIRAD (ISRA-Dakar). Ensuite V. Chevalier est partie de Dakar début 2007 pour le CIRAD de Montpellier, Chauvancy a donc arrêté ses cartes. Je sais que V. Chevalier et F. Baillon se sont rencontrés au CIRAD de Montpellier en 2007 (et 2008 ?) avec un ornithologiste (?) du CIRAD de Montpellier.
- 3// Du fait de mon départ et de mon absence de Dakar en 2008, je sais que des résultats de sérologies d'oiseaux effectuées à l'IPD par A. Sall (sérums d'oiseaux récoltés par l'IRD), ont été transmis directement par l'IPD à V. Chevalier au CIRAD de Montpellier, je ne possède pas ces résultats de sérologies. Comme F. Baillon et V. Chevalier et ont correspondu ensemble en 2007 (et 2008 ?) à propos de ce « volet oiseaux » IRD-EDEN, ces résultats 2008 (couches de migration et sérologies) seraient à rechercher à leur niveau me semble-t-il.

Bien cordialement
Dakar, le 25 Juin 2009

Dr J-F. MOLEZ
Chargé de recherche IRD, MD, PhD

Ampliation : Jean-Marc Hougard
Représentant IRD au Sénégal

WP AFR 4 – Animal reservoirs

Work package number	AFR4	Start date or starting event:			49		
Participant id	IPD	ISRA	CIRAD	IAV	IRD		Total
Person-months / participant	3	2	4	1			10

Objectives

The global objective of this WP is to qualify and quantify the routes and patterns of introduction in Europe of hosts and / or reservoirs (livestock for RVFV, wild birds and their ectoparasites for WNV) from endemic areas, eventually through "bridge ecosystems" in West Africa (Senegal) and Maghreb (oasis, coastal wetlands of Morocco). For the next 18 months, the specific objectives are:

- To finalize the identification of key determinant of local disease spreading;
- To update the description of regional and transcontinental movements of hosts (ruminants, wild birds).

Work performed during previous reporting periods

Investigations on animal mobility in the Senegal River Delta have been conducted in study sites. Questionnaires were established and tested with local veterinary agents. Investigations on the field have validated the approach. These data are currently imported in a specific database linked with spatial models already developed. For wild birds, a database on bird ringing (from the Natural History Museum of Paris) has been made available and some maps on wild bird migrations between Europe and West Africa have been drawn.

Year 3: IRD has continued to monitor wild birds to capture a wide panel of different species and prepare batches of sera samples to be analysed. Birds have been captured using Japanese nets to mesh 12, 19 and 30 mm. Nets were placed from 6.00 to 12.00 am and from 4.30 to 7.00 pm. A total of 757 sera samples were collected in Barkedji (369) and Djoudj (388). These sera samples will be analyzed by IPD virology team. Ectoparasites have also been collected on the birds before serum sampling.

Three types of surveys were performed by ISRA team to describe animal movements in the Senegalese river delta. They took place on market places, in veterinary offices and in the rural area of Ross Bethio. More than 12,000 livestock movements were recorded, of which 82% were related to small ruminants, 16.8% to cattle, 7% to camels and 0.2 to donkeys and horses.

Twenty-two temporary settlements were recorded around Ross Bethio, out of which only 7 represented 72% of the herds investigated.

In Morocco animals movements have not been studied because of difficulties met to collect the required information from the veterinary services.

Animal movements have been studied as usual in Senegal and additional data still in a crude format is available is being analysed and first results presented in Marrakech this trend will be continued No additional data are needed for the Senegalese part of the border.

This work was unfortunately not done in Mauritania and especially in Morocco where the same reasons (difficulties with veterinary services) resulted in the same situation. It might require a special direct discussion of the platform coordinator with veterinary services this must be done quickly for the time remaining in the project.

Description of work

If budget available platform coordinator along with project coordinator should have special direct discussions in Mauritania and Morocco veterinary services to make them confident about the study and set a protocol together.

The animal movements' database available for ISRA and CIRAD on the data management website will be implemented with the new data harvested in 2008. A report on the general pattern of animal movements' will be prepared. Usual describing statistics will be used to determine the different profile of animal movements and the different scenario of risk for the maintenance, the spread and the possible introduction of RVFV in Northern countries.

Deliverables

D AFR 13 - Study report on the links between RVF cases and foci at the Senegal river scale (M38). Achieved. Work presented at the annual general meeting in Marrakech (January 2009).

D AFR 20 - Virus variability between foci (spatial and temporal) for RVF and WNF (M36). Achieved. Work conducted by a PhD study of IPD, and presented at the annual general meeting in Brno (January 2008).

D AFR 21 - Description of regional (ruminants, birds) and transcontinental (birds) movements of hosts and reservoirs (M67). In progress for ruminants (paper to be submitted in 2009). Not achievable for birds: see IRD comment in previous section.

D AFR 22 - First attempts for spreading models (M67). Cf. D Afr 25.

Milestones and expected result

M AFR12 - Potential explicative parameters of the relationship between foci in Senegal and Mauritania are identified and mapped (M48).

To infer from phylogenetic analyses devoted and done by IPD.

M AFR16 - A dendrogram for RVFV and WNV is drawn and describes the genetic similarities between strains from various regional areas. (M48): more virus isolations of virus are needed.

To infer again from phylogenetic studies devoted and done by IPD.

M AFR17 - Ruminant densities and movements in Sub-Saharan and Northern Africa are quantified (M48).

Shortcoming for this has been reported.

WP AFR 5 – Data management and cross disciplinary modeling

Work package number	AFR5	Start date or starting event:				49	
Participant id		ISRA	CIRAD				Total
Person-months / participant		1	6				7

Objectives

The objective of this WP is to provide, through risk analysis and modeling tools, indicators and scenarios to anticipate the risks of introduction, emergence or re-emergence pertinent for the European, regional and national warning and surveillance systems.

For the next 18 month, the specific objectives of this WP are:

- To finalize the multi-sources data standardization and format for latter analysis;
- To test the conceptual model.

Work performed during previous reporting periods

Data from entomological/acarological surveys, movements of animals have been transferred to the database manager. They are currently being imported to the GIS-DB in which the environmental layers are already included (thematic classification of the Senegal river valley and typology of hosts habitats). The conceptual model has been established and presented to the EDEN community during the HIT workshop on modelling.

Year 3:

Birds layer

Connection between habitats map / Birds DB (collaboration between HRRS and IRD teams)

The bird database (Access) has been connected to a GIS (ArcGIS) to map:

- the distribution for each species

- the number of potentially present species in the zone
- the abundance index for each species, and an average abundance index

Mosquitoes layer

Mosquito database (collaboration between IRD and ISRA teams)

A mosquito database has been designed and implemented:

- Species distribution according to habitat, as available from the map of bird habitats.
- Mosquito species present in Senegal (vectors of RVFV or WNV) (review)
- Mosquito activity (experts' opinion)
- Mosquito habitats (experts' opinion)

The links between these different tables have been determined using experts' opinions. A first field validation will be done.

Mosquito maps

The second approach was the mapping of mosquito habitats. Two different methods have been used:

- Apply a buffer (500 m) around all water bodies and analyse the possible habitats inside these buffers: non–supervised image classification.
- Supervise the classification using entomologists' opinion, with birds' habitats as a starting point.

Year 4:

Concerning WN study, we focused on the analysis of environmental risk factors of WN infection in the Senegal River basin. Two Landsat images were process to map the land cover of relevance for host and vectors and WNV (in collaboration with CIRAD and IRD teams). The proportion of each land cover type was calculated for each of the 5 study areas where a survey on WNV seroprevalence in horses was conducted. Serological data were analysed using a generalized linear model, with the individual serological status as the response. As the surfaces occupied by the cover types within a buffer were highly correlated, a Principal Component Analysis (PCA) was carried out to synthesize the initial information on the landscape into independent factors, i.e. Principal Component (PC). These factors were included in the model as the explanatory variables. Results highlighted a significant heterogeneity of prevalence between the study sites. PC1 and PC4 were statistically linked to the serological status ($p = 8.10^{-4}$; $p= 0.04$) suggesting that the components of the first principal component - water, grassy vegetation and inundated during rainy season "tan" - were protecting factors whereas the main component of PC4 – cultivated areas such as sugar cane - was risky factors for WNV transmission.

Modelling studies on RVF vectors focused on the development of a hydrologic model using remote sensing data to describe the dynamics of ponds in the Ferlo area and a model of mosquito dynamics.

We used a Quickbird image to identify the ponds, which are the breeding sites for the mosquito vectors of RVF, in our study area (13x13 km around the village of Barkedji, Ferlo region). Then, a "Surface-Level-Volume" model was implemented in a GIS to model the shape of the ponds after a calibration phase using a very high spatial resolution DEM. Finally, a hydric model (Puech, 1992) was applied to model the ponds dynamics (input: daily rainfall; outputs: Surface, Level and Volume of the ponds). We used a dataset of water level data daily collected during 2001, 2002 and 2003 rainy seasons to calibrate and validate the model.

Based on this pond dynamics model, a model of population dynamics of *Aedes* and *Culex* mosquitoes was developed.

On the other hand, the dynamics of host for RVF was modelled using the available information on herds and pasture management (bibliographic synthesis, expert opinion, field data) using GIS functionalities.

Description of work

In collaboration with the data management horizontal team, propositions are currently submitted to the different collaborators of the African-platform to solve the data sharing issue.

The Animal movement database is already on the data management website and is available to ISRA and CIRAD. Then at the end of the project it will be available to all the members of the Africa platform. Contact details will also be posted with the data. The data would be available to others only on request

after contact (and agreement) with the relevant data owners. Environmental data (land cover maps) are already available for all members of the Africa platform on the data management website.
The Platform database template will also be posted. This would inform other partners within the Africa platform what has been collected and allow them to record there data using the template if required.
Data summaries and contact information will be provided of the Serology and other relevant data-sets that are currently still sensitive due to pre-publication. These data summaries will be available to the public unless requested otherwise. It is suggested that these data summaries would include the information outlined below at the very least. It is possible to post or link to documents if there are documents available which would help describe the data.

- Dataset Name
- Description/summary
- Data contact (Name email)
- Institute
- Number of records
- Variables recorded (or state reference the template).
- Date of data collection

Valorization: the results of the different studies and models will be published (1 publication submitted, 2 in prep.).

RVF: Quality assessment of the vector-model will be achieved using entomological data from a previous project (2002 data) or EDEN data if available.

All sub-models (ponds, vectors, hosts) will be implemented into a GIS environment. The validation of the models of vectors and hosts dynamics and the integration between vector and host models will be achieved.

The re-emergence processes of West Nile virus (WNV) in Europe are currently tested. Infection dynamics were modelled using a SEIR model for bird populations and a SEI model for vector populations. The outputs of the model were the predicted seroprevalence rates on wild birds, as well as the predicted incidence rates on horses, on three locations: a wet African area where WNV is supposed to circulate all year long (Senegal river valley, Senegal, area 1), a dry African area where WNV is known to circulate only during the rainy season (Ferlo area, Senegal, area 2), an European area where WNV may be introduced by migratory birds (Var area, France, area 3. The calibrated model reproduced accurately the observed data, and suggested that palearctic migrations are necessary for a perennial WNV circulation to take place.
Animal movements modeling and related with RVF outbreaks data permitted to explain the dynamic patterns in the emergence of the disease. Some recorded movements of animals during the years 2006-2007, were analysed descriptively using Social network analysis (SNA). SNA and graph theory provide a tool to organise and analyse relational data overcoming the limitations of standard methods where the position of individuals/observations does not affect the result of the analysis. We used SNA to study the trade patterns of animal movements in a specific context such as pastoral areas with nomadic pastors (Senegal valley river). With the data available, a directed dichotomized network with nodes and arches was realised. Relations (relative betweeness, k-neighbours and structural equivalence) between nodes were analysed in association with prevalence data. Dynamic of the outbreaks were related to the movement patterns. This approach allowed visualization and analyses of different levels of organization that existed. Creation of a path for potential transfer of pathogens could be stemmed from this movement network.

Deliverables

D AFR-23 - Integration of remote sensing data for monitoring of key environmental parameters (M48). Achieved.

D AFR 24 – Importation of the available field data and remote sensing data (M48). Achieved for the first data. This Is being finalised by CIRAD pending delivery of field data by partners.

D AFR 25 – First reports of simulations in the conceptual models (M54). In progress. The next step of the infection dynamics model for WNV circulation will be to test several climatic scenarios and the influence on the virus circulation. These results will be presented at the ISVEE congress in August 2009.

Concerning the model of the spread of RVF in the area of Barkedji, using an agent-based model. The conceptual model was built and the computerized model should be implemented within the end of 2009.

Our first results on the dynamic patterns in the emergence of RVF in the Senegal River delta will be presented at the ISVEE congress in August 2009. Social network analyses will be extended to both markets and camps data to provide an overview of the risk for RVF to spread in and out Senegal.

Milestones and expected result

M AFR18. The GIS is developed and include the metadata of the conceptual model (M54).

Achieved. The different information layers are already available in a GIS environment. Results of modelling studies (RVF, WNV) will be integrated in the GIS as dynamic risk maps.

Workpackages horizontal integration and management

WP 6.1 Horizontal data management

Work package number	WP6.1	Start date or starting event:				37
Participant id	Euro-AEGIS					Total
Person-months / participant	14					14

Objectives

The general objective of the data-management work package is to develop web-based, user-friendly data management tools and information systems needed to achieve the objectives of EDEN. The specific objectives during the second 18 months period are:

1. The further development the web-based Integrated Data Exchange and Archiving System (IDEAS v2.0) termed DMT website to assist the EDEN community in developing predictive emergence and spread models including; a) a user friendly web portal providing secured access to data archive and web resources, b) the continued strengthening of the spatial data archive, and c) an EDEN PhD web section.
2. Technology transfer and training through an improved communication network within and beyond EDEN.
3. To prepare for the development of a disease information system to be developed in the last two years of the EDEN project.

Work performed in the previous reporting periods

The first three years of the EDEN project has seen the Data Management Team establish two independent websites – one a major data archive for use by all EDEN members, and the other a site requested and used by EDEN PhD students. The data website now contains a huge volume of standardised spatial data and for use in disease modelling studies, as well as a wide variety of Sub-Project specific information for use within particular Sub-Projects.

A substantial range of information has been specifically prepared by the DMT in response to SP requests, using its in house expertise in data processing and geographic analysis.

The sites are highly visible and attract an encouragingly numerous and diverse range of visitors from inside and outside EDEN. Interest from other Projects for integration and collaboration is beginning to materialise.

The complexity and extent of the established websites means that an increasing proportion of available resources are being used for site maintenance and updating and hardware may have to be upgraded to cope with the demand.

Overview of recent achievements:

a) Maintenance and Servicing existing products and collaborators

Both the main Data-site and it associated EDEN-PhD site continue to expand in terms of membership, search engine visibility, and use. This requires significant resources to maintain and service. A new server was purchased and installed in June 2008, and the old one reconfigured as a backup. The site is thus now both faster, and more reliable. Combined usage regularly exceeds 150 unique visitors a day which is remarkable for a specialist technical site. A high proportion of these visitors are from outside the EDEN community, which means that the site is significantly enhancing EDEN's visibility and profile to the 'outside world'. The success of the EDEN Phd Site in particular has significantly added to the training element of the DMT activities.

b) Expanding provision of standardised georeferenced datasets to Data-site Members

A wide range of new datasets have been provided for the users, many of which are in the throes of detailed analyses of the data collected during EDEN's early years. These include: EU wide trees species distributions (specifically sourced for ROBO and TBD subproject members); remotely sensed indices of vegetation phenology; revised Administrative Unit boundaries at several levels for all EDEN countries; Climate change projections for temperature and rainfall from a number of sources, one in collaboration with the ECDC-funded V-borne project; new high resolution land use land cover layers; Mammal Biodiversity mapping outputs; Soft tick spread models to Europe. There are now nearly 900 datasets in 90 data groups in 20 categories.

c) Preparation of final outputs due in 2010

Subproject data continues to expand at only a modest pace – with new information for TBD and LEISH subprojects. It has become very evident that sharing raw project data (particularly disease related information) through a central site is acceptable to only the closest collaborators. As a result the emphasis of the subproject data sharing has been moved from the exchange of sensitive data between relatively few researchers to the widest possible dissemination of what data have been acquired, from where, using what methods, and by whom, alongside a summary of any results that have been published. The intention is to describe and draw attention to the researchers and their data resources, without endangering their intellectual property rights, so that potential collaborators or indeed funding bodies can identify who best to approach for certain kinds of information and expertise. This searchable, georeferenced and mapped database is being compiled as one of the main components of the EDEN Information System that is a primary output of year 5.

d) Preparing for the continuation of the data archive facility beyond the end of the project

Efforts have been in train for much of 2008 to find ways to extend the life of the EDEN Datasite beyond the end of the project, so that its content continues to be available to the network of researchers that has been developed by the EDEN approach. A number of possibilities are under discussion including the adoption of the site by the ECDC in Stockholm. This would include the active advisory involvement of the ECDC in determining the details of the final DMT outputs due in 12 months time

Description of work

For the final twelve months the DMT will have the following six primary objectives:

f) Maintenance and support of the current websites and data archives. Given the increasing complexity of these products, this is likely to require a substantial proportion of the available resources.

g) Identification and addition of data to the website. This will include public domain data, as available, and EDEN data as provided by the Sub-Projects

h) Provision of technical assistance and advice for GIS and data management to EDEN partners as requested. Given the level and number of requests received to date the majority of assistance will most probably be implemented via email replies to specific questions and queries.

i) Epi-GIS distance learning tool.

j) Production of the EDEN Information System

   a. EDEN scientific data spatial information system (continued as started)

   b. EDEN ecosystem at risk spatial information system: The primary aim of EDEN was to: "Identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change, which can influence the spatial and temporal distribution and dynamics of pathogenic agents." Therefore the objective is to development queryable

DVD based GIS tool which enables to query a spatial data archive of selected data to provide answers to questions related to the original EDEN aim. The system will run in two modes: (a) Pre-prepared query outputs with ready to use analysis outputs and (b) Free queries and analysis by advanced users either using the included data sets. Whilst the knowhow to develop such a tool is available within the DMT team, its development and implementation will greatly depend on inputs provided by the SP leaders.

k) Negotiations for the continuation of the EDEN data archive in a permanent location

Deliverables

D DMT08 – Networking at international workshops and conferences to assess EDEN external user needs (continuous activity pending opportunities reported in annual report) (M60-M67).

D DMT09 – New epidemiological EDEN SP data sets, and new 'upon request' data sets from internal and external EDEN users are included in EDEN DMT website (continuous activity pending identified needs reported in annual report) (M60-M67).

D DMT10 – Provision of value added data processing to assist with interpretation of EDEN outputs (continuous activity pending specific requests reported in annual report) (M60-M67).

D DMT15 – Contribution to discussion on EDEN disease information system at the EDEN SC and AG meetings, and meetings involving international user organizations (continuous activity pending meetings organized by coordination reported in annual report) (M60-M67).

D DMT17 – GIS module included in Moodle distance learning environment (M62)

Milestones and expected result

M DMT03 - EDEN partners request DMT assistance (continuous).

M DMT04 - International workshops and conferences are identified to enable proper networking and identification of external user needs (continuous).

M DMT08 – Partners contribute necessary information to develop 'eco-system' part of information system (continuous)

WP 6.2. RS Tools

Work package number	WP6.2	Start date or starting event:				37
Participant id	ZOOX	UCL	CIRAD			Total
Person-months / participant	16	18	25			59

Objectives

The general objectives of the RS-Tools Work Package are (i) to provide the EDEN Sub-Projects with both high- and low-resolution satellite imagery products, (ii) to train and advise EDEN members on the use of satellite imagery for the monitoring of environmental changes and their application to the dynamics and distribution of vectors and vector-borne diseases and (iii) to quantify through spatial modelling the interactions between environmental changes and disease risk.

According to the Project Document (Annex 1, 6.C Milestones), the major milestones to work towards in this reporting period (Months 25 through 42) are as follows:

- M24 – First generation epidemiological models of selected diseases and pan-European eco-climatic time series analysis are available and enable the development and fine-tuning of second generation models for each of the selected EDEN diseases.
- M36 – Fine-tuned second generation epidemiological models of selected diseases are available to identify factors and indicators of change (both disease-specific and generalised) and pave the way for generic health / environment models. Results are presented at an international conference organised by EDEN.

The three HRRS HIT operational modes:

4) Service Mode, for the provision of land use and land cover and land use and land cover change maps to Sub-Projects.
5) Collaboration Mode, working with the Sub-Projects, carrying out multivariate statistical analyses linking vector density or disease incidence/prevalence to landscape scale environmental variables.
6) Land Use Research Mode, to develop new techniques for the integration of land use and spatial heterogeneity variables in epidemiology, at the landscape level.

Corresponding objectives are:

- Acquisition of high-resolution images for the study sites of the Vertical Sub-Projects and update the EDEN data website accordingly (service mode)
- Processing of high-resolution images for the study sites of the Vertical Sub-Projects, for the production of land cover/land use and land cover-/land use-change maps (service mode)
- Quantitative analyses of vector and disease database with landscape variables derived from the land cover/land use maps produced and other landscape-level data sources, with EDEN partners (collaborative and research modes)
- Production of geo-databases of vector, hosts and ecological conditions (collaborative mode)
- Agent-based modelling of human-environment interaction in the context of disease transmission (collaborative and research modes)

Specific LRRS HIT objectives for the 18 months period beginning project month 25:

Planned exchanges with EDEN partners include:

- Site visit to Senegal (Thiongane et al) (EDEN AFR)
- Site visit of EDEN ROBO (Finland, Henttonen et al) to ZOOX4
- Continued interaction with EDEN ROBO (Sweden, Olsson et al)
- Continued interaction with EDEN ROBO (Leirs et al)

- Site visit of EDEN LEISH (Ready et al) to ZOOX4 or v.v.
- Continued collaboration with EDEN TBD (Randolph et al).

The LRRS HIT operates in four modes (Project Document Annex 1, BLOCK 2 Horizontal Integration, Work Package 6.2, p. 38 – see also box below).

The four LRRS HIT operational modes:

5) Service Mode, for the provision of processed multi-temporal data to Sub-Projects.
6) Collaboration Mode, actively working with the Sub-Projects to generate statistical models of the distribution and abundance of both vectors and diseases using a variety of multivariate methods (mainly based on discriminant analytical, maximum-likelihood methods) and remote sensing data, and to produce biological or process-based models for those vectors and diseases for which sufficient data exist, or will be collected during EDEN.
7) Research Mode, to develop new techniques for the integration of the spatial information from high-resolution satellite data with the temporal information from multi-temporal satellite data.
8) Research & Service Mode, actively working with partners to develop techniques for detecting changes in habitat seasonality over time – a guide to detecting whether habitats have changed from the perspective of the vector-borne diseases within them. A secondary objective here is to establish the correlations between the old (e.g. AVHRR) and new (e.g. MODIS) satellite data time series.

Applicable to respective modes specific LRRS HIT objectives are:

- To continue to download MODIS and other data as appropriate, to establish contemporary pictures of European habitats (service mode).
- To continue to process the downloaded imagery and to update the EDEN website with processed data (service mode).
- To apply information-theoretic statistical models to the datasets provided by EDEN partners to date (collaboration mode).
- To begin to develop satellite-driven biological models for selected EDEN vectors (collaboration mode).
- To investigate with the HRRS partner 4 (UCL) the integration of low- and high-resolution imagery for selected EDEN field sites (research mode).
- To investigate continent-wide seasonality changes over time, as detected by the AVHRR time series; are they artefactual or real? (research and service modes).
- To continue capacity building and technology transfer (all modes).

Work performed by HRRS in the previous reporting periods

As part of the 'environmental change' (HRRS) HIT, we continued to work closely with all sub-projects. With each sub-project, we conducted one or several empirical data analyses on a specific disease, in a specific region or country, and on a dataset collected by sub-project partners. All these projects involved high resolution satellite imagery for land cover classification and change detection, and modelling of these data with vector, host or human data in a spatially-explicit way. We modelled vector/host presence-absence, abundance, and serological prevalence, as well as human cases of infection.

We continued to collaborate with MAL to test some scenarios for land use changes and their impact on the risk of re-emergence of malaria in the Camargue area (France). Results indicated a low risk in the area.

We collaborated with TBD on landscape determinants of TBE infection, with a focus on land use and land tenure. We collaborated with ROBO on ecological factors associated with rodent trapping data.

Collaboration with WNV teams implements a study on the distribution of mosquitoes *Culex modestus* and *Culex pipiens* and environmental variables and the use of GIS to map the areas the more at risk for WNV circulation according to different hypotheses on the factors involved in the introduction/amplification/spread and emergence of the virus.

Collaborations with AFR-Senegal teams continued for the study of WNV in the Senegal Delta: an analysis of environmental risk factors of West Nile infection in the Senegal River basin was performed based on Landsat imagery. The dynamics of hosts and vectors of Rift Valley Fever were modelled in a GIS environment.

Eric Lambin attended the scientific committee meetings, and HRRS group members attended several meetings with vertical project colleagues (in Montpellier,…), presented our work at international scientific meetings and produced several scientific publications.

Description of work HRRS

UCL-Finland (ROBO): Statistical analyses will be performed to relate environmental remotely-sensed data with bank vole abundance and Puumala hantavirus prevalence data.

UCL-Sweden (ROBO+TBD) : Statistical analyses will be performed to relate environmental remotely-sensed data with incidence data of tick-borne encephalitis

UCL-Liverpool (ROBO): Statistical analyses will be performed to relate environmental remotely-sensed data with bank vole abundance and cowpox virus prevalence data.

UCL - NHM (LEI) - UU (MM) – ZOOX (LRRS): Using the example of sandflies and leishmaniasis, integration across horizontal teams will be investigated by the construction of biological models relying on high- and low-resolution data.

CIRAD-EID-IRD (WNV): the risk areas identified according to the different scenarios of introduction/amplification/diffusion/emergence will be compared with areas of virus circulation (equine cases, seropositive birds) during the last years.

CIRAD-DDI-IZS-ISRA (WNV/AFR): A comparative landscape analysis will be performed on four sites with WNV circulation (Camargue, Danube, Padule di Fucecchio, Senegal River).

CIRAD-ISRA-IPD (AFR): Dynamics models of RVF vectors and hosts will be integrated and analysed.

In addition to analytic and modeling work, we will focus on the publication of the results from previous years.

Work performed by LRRS in the previous reporting periods

The LRRS HIT team has downloaded and processed multi-temporal satellite data, examined changes over time in the longest time series of multi-temporal data available (from the AVHRR sensor), and developed statistical risk predictions using datasets from EDEN-ROBO, EDEN-LEI and EDEN-TBD. A new information-theoretic method has been explored to identify key variables, and a new way of integrating point and polygon PH data has been formalized.

This work involved collaboration with the EDEN SPs named above, and as detailed earlier in this Report.

In addition, a considerable amount of time and energy has been spent in trying to ensure some sort of future for the ideas and activities generated by the EDEN project. The EDEN Secretariat, and specifically Guy Hendrickx, is to be commended for its many activities in this area, that have involved vists to numerous institutes and organizations across Europe.

Description of work

ZOOX-Sweden (ROBO): Statistical analyses using low resolution MODIS data applied to point records of TBE and Tularemia cases, and identification of key variables

ZOOX-UA (ROBO): Statistical analyses of hantavirus data for several European countries

ZOOX-ZOOX (TBD): Statistical analyses of TBE data for Hungary and other countries, and production of TBE risk maps.

ZOOX-NHM (LEI): Continuing collaboration of risk mapping of both leishmaniasis and its vector in Europe.

ZOOX-UCL (HIT): Collaboration on lo- and hi-res image fusion using a variety of image-furion techniques.

ZOOX-HITs and Selected SPs: Integrated modeling using lo- and hi-res imagery, mathematical models and selected field data sets.

ZOOX: Analyses of trends in the MODIS time series 2001 – date, using moving TFA windowing techniques, as previously applied to the AVHRR time series.

Deliverables

D HRRS01 – High resolution image analysis for each selected site (M60) Ongoing, status updated in each annual report.

D HRRS02 – Landscape analysis and environmental change analysis for selected HR sites (M67) Ongoing, status updated in each annual report.

D HRRS03 – On the job HRRS technology transfer (M67) Ongoing, status updated in each annual report.

D LRRS01 – Fourier processed LRRS MODIS data archive established and updated (to M60) MODIS archive to be updated with the 2001-2007 data. A complete time series of MODIS v5 processed images will be completed

D LRRS02 – Pan-European maps of eco-climatic seasonality signals (M60) Unsupervised classification of Temporal Fourier processed MODIS imagery.

D LRRS04 – Information-theoretic models, including identifying key predictor variables, for more of EDEN diseases (M67)

D LRRS05 – On the job technology transfer (M60) Hosting visits from SP teams, and visits to field and other sites.

D LRRS06 – Process-based vector models for candidate diseases (depending upon field data availability) (M60). Study will be conducted provided seasonal datasets of univoltine or multivoltine vectors within Europe are available.

D LRRS07 – Data-fusion techniques for selected imagery developed (M60).

D LRRS08 – Analysis of changes detected through moving windowed Fourier techniques (M67).

Milestones and expected result

M RS18 – MODIS data are Fourier processed and available to be included in spatial models (M60). First iteration (MODIS v4, 2001 to 2005) available, second iteration (MODIS v5) time series now being processed on UK Grid computers.

M RS19 – Strategy document enables development of integrated approaches of risk assessment linking high resolution information with low resolution surrogates of risk (M60). Strategy Document re-visited at Brno AGM and updated. Will be updated further in the light of experience now gained about the feasibility of DMEWS in the future.

M RS21 – Synthesis of empirical findings on linkages between the different agents responsible for disease transmission (vectors, hosts, humans, environment) (M60).

M RS22 – Integration of several HITs and selected SPs to leverage EDEN's results to maximum effect.

M RS23 – Development of a post-EDEN strategy for EDEN activities, results etc., especially concerning the future of EDEN's databases (M60).

The above will lead to joint publications that present a synthesis of empirical studies on different diseases, in different study areas, and the identification of useful tools for a future DMEWS.

WP 6.3 – Horizontal PhD Program

Work package number	WP6.3	Start date or starting event:		37		
Participant id	UCL	FVM	IRD				Total
Person-months per participant	18	18	18				54

Objectives

The overall objective is to train EU students to PhD level in a variety of advanced, multi-disciplinary analytical techniques and to spread knowledge of these techniques throughout the EDEN community. Topics include epidemiological modeling, environmental change modeling and community ecology of infectious diseases.

The specific objectives of the next 18th months periods are:

- For the 'Risk maps and Disease Modelling' PhD: to construct generic epidemiological models for vector transmitted disease agents and geographic maps for such systems;

- For the 'Environmental changes at the landscape scale' PhD: to analyse the effects of land use change on the distributions of disease vectors and vector-borne diseases;

- For the ' biodiversity and health ' PhD: to evaluate the impact of the biodiversity (in term of local and regional vector/reservoir species richness and composition) on locally infectious disease transmission.

Work performed in the previous reporting periods

Students were selected for PhD on the following research topics: Risk maps and vector modelling; Environmental change at the landscape scale; the linkages between biodiversity and health. The students joined the different workshops (Data management, GIS and Remote Sensing Workshop, Mathematical Modelling workshops, PHD Meeting) and contributed to the discussions on a complementary approach. In addition, meetings between HIT PhD and vertical SP teams have been organized. Maintaining this network will be an important objective of next reporting period.

MatMod: Two scientific papers are in press in respectable scientific journals: one is collaboration with the West Nile Virus team, the other with the TBD team. A third manuscript, where the spatial methodology for risk mapping is developed, is collaboration with the Low Resolution modeling team. Meetings have been held with the Leishmania team and the High resolution team. This will lead to collaboration on risk maps for Leishmania in the coming period.

In the third period we collaborated with the Leishmania vertical team (will be concluded in the final accounting period with a joint publication) and we started collaboration with the malaria vertical team. The work with the Leishmania team for the first time combines the expertise of a vertical team with that of three Hits (Mod, LR and HR). Methodology was developed using BTV as an example (due to problems with Leishmania data that have now been solved).

BioDiv: A first paper has been published and an additional series of papers are in preparation using results obtained to date. Collaborations are ongoing with the EDEN WNV and HRRS teams and scientific exchanges were organized with other PhD students: N. Ponçon (SP MAL), G. L'Ambert (SP WN) and K. Tersago (SP Robo). In addition data on West Nile virus, avian flu and Lyme disease originated outside EDEN were used to develop modeling approaches and test hypotheses.

During the last 12 months term, HIT BioDiv activities have concentrated their efforts on the writing of scientific publications based on results acquired from previous years. In particular, HIT BioDiv Benjamin Roche' PhD student passed with honorific distinction last June 2008, 27th his oral defence at Montpellier University, and he got a short term employment contract at FAO in Roma to work on avian flu dynamics (FAO proposed him a long-term employment contract he refused), and he is now post-doctoral fellow at Athens University (starting date: 08/01/08). Overall nine papers have been written during this term, 3 of them are book's chapters (none are EDEN-referenced) and 6 are scientific publications (3 are actually EDEN-referenced and 1 will be soon).

One paper (papers' ranking below follows the present description) has more specifically studied the impacts of biological diversity and its species composition upon the circulation of some pathogenic agents. Here, in both using theory and data (West Nile Virus, LP avian flu virus, Lyme disease), we have shown what can be the impacts of community species patterns and its heterogeneity on disease transmission for both zoonotic and vector-borne diseases. Lyme disease, low pathogenic avian flu viruses and West Nile fever in real conditions have been compared with models issued from our study.
A second work has focused on the impacts of host bird species reservoirs composition, diversity and dynamics on low pathogenic (LP) avian flu viruses in Southern France. In this investigation, 3 scenarios of LP avian flu viruses transmission have been tested, ie. by migratory birds, by the aquatic environment and by direct contacts between bird individuals. Results suggest, in both using mathematical modelling and an impressive data set for both bird species and avian flu virus types, that the transmission patterns driving the dynamics of infection in the bird sanctuary Camargue delta involve both a density-dependent and a water-borne transmission. However, water-borne transmission appears to be the main determinant of the disease dynamics and observed prevalence level, thus rejecting the hypothesis of a transmission of these kinds of virus through transcontinental bird migrations from Africa to Europe.
A third work has focused on the effects of space and its heterogeneity on disease transmission in proposing a new type of computational modelling, and we developed a multi-agent based system model for vector-borne disease transmission in a realistic spatial environment. This work is available as a free-software on the net. Of the same vein, a fourth paper has more deeply analyzed the spatio-temporal dynamics of vector-borne diseases in heterogeneous patchy landscapes. In this study, we focused on one particular aspect of disease behaviour in fragmented landscapes, which is disease (un)predictability to emergence and spread. The question of the advisability of opting for a certain type of disease control policy when facing unpredictable disease behaviour has then been discussed.
A fifth paper has studied the influence of trophic chains and ecological interactions between species and individuals on disease transmission from both a pure theoretical side and a more empirical one. The empirical study is an extra from EDEN, and this is the main reason why we did not ask for an EDEN number for this paper. Since the philosophy of this work is EDEN-based, we then present it. Using mathematical modelling supported by quantitative data from 27 different localities in Western Africa, we have developed an optimal ecological web model which represents how Mycobacterium ulcerans agent might be transmitted within local aquatic host communities from host individual to host individual. Besides its applied aspect for the understanding of M. ulcerans ecology, this study underlines the efficiency of parasite transmission through ecological webs, a notion which could be highly relevant also to many other kinds of infectious diseases.
A sixth paper, to be finished soon, has used a comparative phylogenetic analysis to show the existence of a negative relationship between average body mass of bird species and their seroprevalence for West Nile virus in captive avian species. These findings suggest the existence of an allometric relationship between body mass and intrinsic susceptibility across bird species that it could be explained by the increase of immune system complexity with body mass in bird species. This work will facilitate the understanding of West Nile virus transmission in wildlife, and for which the body mass of bird species seems to be a good indicator of disease seroprevalence. This work is still in progress through a positive collaboration with partners from Cirad and EID.
Three books' chapters (2 in French and 1 in English) have been written, all of them addressing the impacts of global environmental change and their interactions (habitat fragmentation, land use changes, bioinvasions,…) on host-pathogen associations, with major consequences in health ecology (emergence and re-emergence, evolution of virulence and transmission,…). In this series of publications, we demonstrate how biodiversity sciences, epidemiological theory and evolutionary ecology need to cross-fertilize together to better understand disease emergence and transmission, notably when the environment is facing some important perturbations and alterations.

Description of work MatMod

1. Prepare manuscript on methodology integrating LR-Hit and Mod-Hit (BTV as case study) for new journal; 2. Finish work on Leishmania for South of France, integrating LR-Hit, HR-Hit and Mod-Hit for the first time with a vertical team; 3. Finish paper on sensitivity analysis of R0 using Tick-borne diseases as case study; 4. Finish PhD thesis; 5. Assist all vertical teams with modelling and R0-risk mapping

(work with the malaria team has already started).

Description of work BioDiv

Research efforts have concentrated on the development of statistical and mathematical modelling that may optimally capture the potential linkages between ecosystem and biodiversity dynamics, and infectious diseases patterns. First, two categories of modelling have been implemented: on one hand a series of multi-species SIR models, based on the seminal work by Andy Dobson, have been developed that take into account the local population and community dynamics of host/vector species and their impacts on infectious diseases (study-cases are LP avian flu viruses, West Nile Fever virus, Lyme disease) (publication has been submitted); on another hand, an agent-based model has been developed for vector-borne disease. This ABM is sufficiently flexible enough to be applied for a large variety of vector-borne diseases situations. Implementation of specificity for particular situations is also possible. The free software is available at http://roche.ben.googlepages.com (programming language: Java on a Swarm platform). This ABM will be coupled to a GIS platform within the next few months, using West Nile virus in the Camargue area as a study-case (collaboration with A. Tran, G. L'Ambert and others). Finally, through a fruitful collaboration with Cirad partners at Montpellier (N. Gaidet, G. Balança, S. Morand, A. Tran and others), a statistical comparative method has been assessed that takes into account the potential effect of phylogenetic inheritance of some life-history traits across bird species, like the cross-correlation between body mass and susceptibility/competence in reservoir hosts, in the disease transmission lifecycle. Again, West Nile virus disease has been selected as a candidate disease to test the effects of inherited life-history strategies and traits on disease transmission and spread.

Deliverables

D PhD 01 – Respective methodologies published and included in strategy document (M42)

D PhD 02 – MatMod PhD progress report (M52)

Milestones and expected result

M 34 - The discussions at the annual meeting and PhD meeting strengthen the PhD network and contribute to the wider dissemination of the EDEN approach (M51).

WP 7 – Overall integration: tools and scenarios

Work package number	WP7	Start date or starting event:			49	
Participant id	ZOOX	FVM	UCL	CIRAD	IRD	IPP	METLA
Person-months per participant	2	2	2	2	2	1	1
Participant id	NHM	AFR	Euro-AEGIS				Total
Person-months per participant	1	1	1				15

Objectives

The overall objective of this work package is to integrate the information from the Sub-Projects on individual diseases through the activities of the Horizontal integration teams in order to be able to achieve the general objectives of EDEN which are (a) to identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change, which can influence the spatial and temporal distribution and dynamics of pathogenic agents in general and (b) through the development of generic tools to provide support for monitoring systems, predictive emergence and spread models and global and regional preventive early warning systems.

This is achieved through the writing of a RTD strategy document (SD). During the first year a SD draft was written by the central management team based on discussions prior to the start of EDEN, at the Kick-Off meeting, during EDEN integration workshops and at the Steering Committee meetings. In this reporting period the second version will be finalised. The dissemination of outputs will be organized under WP8.

Work performed in the previous reporting periods

Two versions of the strategy document have been produced and discussed. The current status of the different chapters is summarized in the table below.

Chapter 1 – EDEN project	Completed
Chapter 2 – State of the Art per Sub-project and Horizontal Integration Team	Completed
Chapter 3 – EDEN strategy in theory	Completed
Chapters 4-9 – Individual disease chapters	In progress
Chapter 10 – Generic models	Not started
Chapter 11 – Information systems, tools and scenarios	Notes included
Chapter 12 – EDEN dissemination plan	Completed
Appendix A – EDEN strategy in a nutshell	Completed
Appendix B – Elements of a dissemination plan	Completed

The strategy document will be included in the proceedings of the international conference organised at the end of the EDEN project. For this purpose, a general approach will be proposed during the annual meeting 2009 in Marrakech, to plan integrative publications, and how this should build up toward the international EDEN conference and the ongoing initiatives related to the "post-EDEN era". Each SP leader will propose the publication plan of her/ his SP with the focus on integrative publications, and the chairman of the steering committee will summarize the main scientific messages coming out of the results and which should be the focus of the final EDEN conference.

Description of work

- Publication plans per SP including HIT interactions as an outcome of the EDEN annual meeting in Marrakech.
- Discussion of progress made at the two other SC meetings in June (UK) and December (Brussels) 2009. Depending on available resources, a final SCM may be organized in April/March 2010. The main goals of these SC meetings will be to facilitate the integration of the EDEN results, the development of generic models of vector-borne disease emergence, and the categorization of ecosystems at high risk of vector-borne disease emergence.
    - Depending on resources Skype telephone conferences may be organized (instead of face-to-face SC meetings) with sub-groups of the EDEN SC to discuss generic models per disease group.

During the AGM and the SC meetings particular attention will be given to the organisation of the international conference at the end of EDEN (May 2010), to present and share the most prominent and integrative results to the international scientific community.

Deliverables

D OI05. Final version strategy document[(*)] v3.0 (M67).

* The final version of the strategy document will be the proceedings of the international conference organised at the end of EDEN.

Milestones and expected result

M OI04. The SP publication plans are available at the end of the EDEN AGM in Marakesj. (M51)

M OI05. The SP and HIT leaders contribute ideas at the various SC meeting to identify inicators of change and develop generic models (M51–56–60–64).

WP 8 – Management, training & dissemination co-ordination

Work package number	WP8	Start date or starting event:				49	
Participant id	CIRAD	ZOOX	FVM	UCL	Euro-Aegis	IRD	IPP
Person-months per participant	28.5	4	1	1	1	6	1
Participant id	METLA	NHM	ISRA	Avia-GIS			Total
Person-months per participant	1	1	1	7.5			53

Objectives

Because EDEN is a complex project, the consortium has opted for a strong central management and compact Steering Committee. The day to day coordination of EDEN is conducted by three persons: the project coordinator focal point to the EU, the chairman of the Steering Committee (in charge of the scientific coordination) and the secretary of the Steering Committee (in charge of the operational coordination). They are assisted by a management team composed of (i) a financial manager, (ii) an administrative assistant, and (iii) timely inputs from specialists (web-master, project engineer, communication specialist, graphic designer etc.). The overall objective of the central management committee is to co-ordinate the management, training and dissemination activities of EDEN.

Work performed in the previous reporting periods

- Implementation of the EDEN website and SP websites
- EDEN leaflet and poster for dissemination
- Kick-off meeting (in Montpellier): 110 participants
- General annual meetings in Finland, Turkey and Czechland, with 130 participants.
- 3 steering-committee meetings per year in 2005 and 2006, 2 in 2007.
- West Nile meeting in Montpellier with 36 participants.
- R_0 workshop in Paris with 125 participants.
- PhD meetings in Paris, Antalya and Brno (50 participants). Organization of a PhD award with a price of 2,000 Euros to attend an international conference.
- Many interviews on radios, TV and articles about EDEN in many media.
- Presentation of EDEN in many international conferences by the coordinator and the SC secretary.

Description of work

- Organisation of the annual meeting in Marrakech, including a steering committee meeting.
- Organisation of two other SC meetings in June (UK) and December (Brussels) 2009. Depending on available resources, a final SCM may be organized in April/March 2010. The main goals of these SC meetings will be to facilitate the integration of the specific results obtained to date, and the production of generic models of vector-borne disease emergence, and the categorization of ecosystems at high risk of vector-borne disease emergence. Skype telephone conferences may be organized (instead of face-to-face SC meetings) with sub-groups of the EDEN SC to discuss generic models per disease group.
- During the AGM and the SC meetings A particular attention will be paid to the organisation of an international conference at the end of EDEN (May 2010), to present and share the most prominent and integrative results to the international scientific community.
- Finalisation of the periodic activity report (4^{th} 12-month period), including the periodic management report.
- Regular management of the consortium, including relationships with the European commission.

Deliverables

D MAN07 – Mandatory report on gender action (M60) In preparation.

D MAN23 – EDEN SC in UK (M54)

D MAN24 – EDEN SC in Brussels (Date to be agreed)

D MAN25 – Final EDEN poster and leaflet (M64)

D MAN26 – Final steering committee meeting in Montpellier (M67)

D MAN27 – Final international conference in Montpellier (M67)

D MAN28 – Final report (activity and management) (M67)

Milestones and expected result

M MAN10 – The third annual meeting strengthens EDEN as a group and enables the establishment of new collaborations between partners (M39).

An evidence of this was the capacity to mobilize EDEN partners to reply to a tender lauched by ECDC: "Assessment of magnitude and importance of vector-borne diseases in Europe".

M MAN11 – The annual PhD network meetings strengthen links within the EDEN PhD community and promote scientific integration in EDEN (M39).

Achieved.

M MAN12 – Inputs at SC and AG meetings enable in-depth discussions on EDEN scientific strategy and contribute to problem identification and solving (M39-48).

Achieved.

M MAN13 – The updated EDEN Dissemination plan takes into consideration EDEN's successes and failures in that field of activities and enables the implementation of an improved dissemination strategy (M50)

Postponed after Marrakech annual meeting (M56).

M MAN23: EDEN SC in UK M56. State of modelling work and preparation of the international conference

M MAN24: EDEN SC in Brussels: M60. State of modelling work and preparation of the international conference

M MAN25: Final EDEN poster and leaflet: M64. Summary of EDEN results and advertisement for the EDEN international conference.

M MAN26: Final steering committee meeting in Montpellier: M67. Summary of EDEN results. Dissemination of EDEN results and appropriation by public-health agencies.

M MAN27: Final international conference in Montpellier: M67.

M MAN28: Final report (activity and management): M67.

ANNEX – EDEN publication list

List of EDEN publications

December 17, 2008

Papers

[1] Atkinson, P.M., Graham, A.J.: Issues of scale and uncertainty in the global remote sensing of disease. Advances in Parasitology **62**, 79–118 (2006). DOI 10.1016/S0065-308X(05)62003-9

[2] Barandika, J.F., Hurtado, A., García-Sanmartín, J., Juste, R.A., Anda, P., García-Pérez, A.L.: Prevalence of tick-borne zoonotic bacteria in questing adult ticks from northern Spain. Vector Borne and Zoonotic Diseases (2008). DOI 10.1089/vbz.2008.0023

[3] Bryja, J., Charbonnel, N., Berthier, K., Galan, M., Cosson, J.F.: Density-related changes in selection pattern for major histocompatibility complex genes in fluctuating populations of voles. Molecular Ecology **16**(23), 5084–5097 (2007). DOI 10.1111/j.1365-294X.2007.03584.x

[4] Bryja, J., Galan, M., Charbonnel, N., Cosson, J.F.: Duplication, balancing selection and trans-species evolution explain the high levels of polymorphism of the DQA MHC class II gene in voles (Arvicolinae). Immunogenetics **58**(2-3), 191–202 (2006). DOI 10.1007/s00251-006-0085-6

[5] Carpi, G., Bertolotti, L., Pecchioli, E., Cagnacci, F., Rizzoli, A.: *Anaplasma phagocytophilum* groEL gene heterogeneity in *Ixodes ricinus* larvae feeding on roe deer in northeastern Italy. Vector Borne and Zoonotic Diseases (2008). DOI 10.1089/vbz.2008.0068

[6] Carpi, G., Cagnacci, F., Neteler, M., Rizzoli, A.: Tick infestation on roe deer in relation to geographic and remotely sensed climatic variables in a tick-borne encephalitis endemic area. Epidemiology and Infection pp. 1–9 (2007). DOI 10.1017/S0950268807000039

[7] Charbonnel, N., Chaval, Y., Berthier, K., Deter, J., Morand, S., Palme, R., Cosson, J.: Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. Physiological and Biochemical Zoology **81**(1), 63–73 (2008). DOI 10.1086/523306

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[9] Davis, S., Calvet, E., Leirs, H.: Fluctuating rodent populations and risk to humans from rodent-borne zoonoses. Vector Borne and Zoonotic Diseases **5**(4), 305–314 (2005). DOI 10.1089/vbz.2005.5.305

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